COLOURATION AND ITS ADAPTIVE SIGNIFICANCE IN SEXUAL SELECTION OF JUMPING SPIDERS (ARANEAE: SALTICIDAE)

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DECLARATION

I hereby declare this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has not been submitted for any degree in any university previously.

Chen Zhanqi

25 June 2015

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SUMMARY

Colourful male ornaments are widespread in animals. These ornaments serve to highlight the fitness of the bearer and are subjected to sexual selection via female mate choice and/or direct male-male competition. Conspicuous body colouration including ultraviolet (UV: wavelengths 280–400 nm) reflectance is present in the males of many species. It has been well established that male UV and human-visible (VIS) colouration falls under direct selection through female choice in many animals, including birds, fishes, reptiles, insects and spiders. However, how UV and VIS colouration originated at first place and evolved remains unknown for any animal. Furthermore, the origin and evolution of colour-based female mate-choice in animals is poorly understood. However, a few limitations to our understanding of the evolution of salticid ornamental colouration still exist, although the role of ornamental colouration in reproductive behaviour of salticids has been intensively investigated. First, previous studies have usually focused on either UV or VIS colours, yet no study has investigated both UV (wavelength: 280-400 nm) and VIS (wavelength: 400-700 nm) colourations together. Second, previous studies have mainly described colours based on qualitative human assessments of colouration simply as blue, red, green etc.. However, each colour can be affected by its total brightness (Qt), which is the total area under the spectral reflectance curve within a specific range of spectral reflectance), chroma (purity or stature of colour) and hue (wavelength at which the spectral reflectance value reaches max in a specific reflectance range). Therefore, it still remains unclear which of these three aspects of colouration play a role in sexual selection, and which one is more likely to differ between the two sexes, or among

very closely species. Third, prior studies have often concentrated on one species, and very few comparative studies have been conducted on both UV and VIS colouration of multiple species. Investigating sexual dichromatism and its adaptive significance across multiple species is necessary to get a clear picture of the evolutionary processes behind of colourations in each sex. A comparative approach enables us to analyse potential correlations between colour differences and possible speciation events when mapped onto a phylogenetic tree in the future.

In this dissertation, I aimed to examine UV and VIS colouration and its adaptive significance in sexual selection and to investigate the origin and maintenance of UV and VIS colouration using jumping spiders of the subfamily Heliophaninae (Araneae: Salticidae) as a model system. To elucidate the adaptive significance of UV and VIS colouration, the origin and evolution of UV and VIS colours, and colour-based female mate-choice in these jumping spiders, I tested several hypotheses: (1) Sexual dichromatism (including UV) may be common in salticidae with conspicuous body colours; (2) Male UV and VIS colours may play important roles in sexual selection; (3) Colour-based female mate choice, once acquired, may be maintained by direct and/or indirect genetic benefits; and (4) Sexual dimorphic colouration partially coevolved (at least) with speciation events in the evolutionary history.

My results showed that: (1) Sexual dichromatism is widespread in jumping spiders of the genus *Phintella* and other heliophanine genera examined; (2) Sexual dimorphic colour plays a role in intersexual communication for tropical region species and this colour not always positively contributing to matting success; (3) Female can make mate-choice decisions based on sexual monomorphic colouration of males in some species, and male colouration is a reliable predictor of matting success and

females preferably mate with males that exhibit similar colouration with themselves; (4) Preference for colourful males allows choosing females to gain both direct and indirect benefits in *Chrysilla acerosa*; and (5) The evolutionary change of hue is closely related the divergence of speciation in the evolutionary history. In addition hue and chroma usually show converse evolutionary patterns in the same clade.

In conclusion, these results indicate that sexual dichromatism is common in both UV and VIS ranges of spectral reflectance in the targeted species. And the dimorphic colours on male can be crucial signals in courtship displaying. However, sexually monomorphic colouration on males can be selected by female as well in some cases. The evolutionary change of sexually dimorphic colour patterns on males may contributes to species divergence because of the importance of these colours in sexual selection.

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CHAPTER 1

General introduction and objectives

1.1 Animal colouration and colour vision

Animal colours are of broad interest in evolutionary studies because they may reflect natural selection and sexual selection and the balance between them (Andersson and Amundsen, 1997; Endler, 1990; Grill and Rush, 2000; Thorpe, 1996; Thorpe and Richard, 2001). There are various aspects of colour. Humans percept colours as brightness, chroma, and hue. Brightness is overall intensity, chroma refers to purity of colour, and hue is defined as the focal colour at a particular wavelength range (e.g., red, green, blue) (see Endler, 1990; Grill and Rush, 2000). Almost without exception, evolutionary theories involving colours such as signalling, mimicry, crypsis, warning colours and sexual selection are almost always based human perception. This may lead to erroneous conclusions (Bennett et al., 1994). Indeed, human visual system is very different from that of animals, whose vision system has photoreceptors that are sensitive to ultraviolet (UV; < 400 nm) wavelengths, to which humans are blind. Many diurnal birds, reptiles and amphibians studied are now known to possess tetrachromatic colour vision (Cuthill, 2006; Cuthill et al., 2000b; Hárosi, 1994; Hart et al., 2000; LeBas and Marshall, 2000; Novales-Flamarique and Hawryshyn, 1994). Tetrachromatic colour visual system is different from that of human in several fundamental ways (Bennett and Cuthill, 1994). First, tetrachromatic colour vision is sensitive to near-UV wavelengths (< 400 nm), but humans can not see UV light (reviewed by Bennett and Cuthill, 1994). Second, many vertebrates have at least four spectrally distinctive cone-types, each locating at different wavelength areas having a sensitivity peak (i.e. ultraviolet, blue, green and red) (Bowmaker et al., 1997). However, humans have only three cone-types (blue, green and red), and most of the mammals have just two (blue and green). UV vision seems to be absent in most mammalian species although the retinas of some rodents possess UV sensitive

receptors (Jacobs et al., 1994; Jacobs and Deegan, 1994; Jacobs et al., 1993; Neitz et al., 1991). Third, tetrachromatic color vision in terrestrial vertebrates contains oil droplets, which connect to cone cells. These oil droplets function as a filter to block certain wavelengths to increase colour saturation, which can enhance animal vision system, colour discrimination and colour constancy (Bowmaker, 1980; Jane and Bowmaker, 1988; Vorobyev et al., 1998). Consequently, human colour vision may be only a part of the tetrachromatic and that even quite different from other animals. In particular, species which appear monochromatic in the human visible spectrum may be dichromatic in the UV. Whilst UV vision extends the range of wavelengths over which animals can perceived, increased dimensionality produces a qualitative change in the nature of colour perception that probably cannot be translated into human experience. Animal colours are not simply refinements of the hues that humans, or birds, see, these hues are unknown to any trichromat. Therefore, this has important consequences for testing evolutionary theories of signalling, crypsis, mimicry, warning colouration and sexual selection involving colour (Cuthill, 2006). Therefore, the evolutionary theories involving colour should be reassessed using an animal eye's point of view. My main aim in this thesis is to test evolutionary hypotheses of sexual signalling and sexual selection involving colour by understanding how animals perceive colour.

1.2 Importance of UV vision

UV vision is common among animals, including fish (Archer and Lythgoe, 1990; Bowmaker and Kunz, 1987; McFarland and Loew, 1994; Smith et al., 2002), reptiles (Ammermüller et al., 1998; Jacobs, 1992; LeBas and Marshall, 2000; Shi and Yokoyama, 2003; Whiting et al., 2006) birds (Bennett and Cuthill, 1994; Burkhardt,

1982, 1989; Finger and Burkhardt, 1994; Yokoyama et al., 2000), and many invertebrates (Salcedo et al., 2003; Tovée, 1995), particularly in insects (Briscoe and Chittka, 2001), crustaceans (Frank and Widder, 1996; Smith and Macagno, 1990), including mantis shrimps (Frank and Widder, 1994), and spiders (De Voe, 1975a; Peaslee and Wilson, 1989; Yamashita and Tateda, 1976). UV vision has been found in some mammals (Jacobs, 1992; Jacobs and Deegan, 1994) as well, but not as common as in the other animals mentioned above.

It appears that UV vision is a general property of diurnal animal species (Bennett and Cuthill, 1994; Cuthill et al., 2000a; Hunt et al., 1998a). Behavioural studies have provided evidence that animals use UV for many purposes, such as distinguishing species, navigation, foraging, inter- and intra-specific communication, and sexual selection (Tovée, 1995). In the past three decades, extensive studies have focused on UV vision in intra-specific communication (Bennett and Cuthill, 1994; Cuthill et al., 2000a; Jacobs, 1992; Tovée, 1995) and UV vision in sexual signalling, and in mate choice in particular are becoming the most hottest study fields, especially in birds (Andersson and Amundsen, 1997; Bowmaker et al., 1997; Maier, 1993; Siitari et al., 2002), fish (Boulcott et al., 2005; Kodric-Brown and Johnson, 2002; Marshall et al., 2003), and reptiles (Fleishman et al., 1997). In contrast, we only poorly understand UV vision and its adaptive significance in invertebrates (Tovée, 1995). In this thesis, I investigate how invertebrates perceive UV and their UV perception in sexual selection with focus on female mate-choice using jumping spiders (Araneae: Salticidae) as a model system.

1.3 Sexual dichromatism

Sexual dimorphism, a phenotypic difference between the sexes within the same species, is widespread across animal kingdom and evolves when particular traits are subjected to selection (Campbell, 1972). Numerous studies have shown that sexual selection is the major driving force of sexual dimorphism in a wide range of species (Andersson, 1994). Many animals show sexual dimorphism in colours termed as 'sexual dichromatism', which refers specifically to the difference in colouration between males and females within the same species. Sexual dichromatism is widespread in animals and often attributed to sexual selection for traits in one sex that reflect light as a conspicuous visual signal to members of the same or opposite sex (Andersson, 1994; Houde, 1997; Silberglied, 1984). However, the association between specific sexually dichromatic colouration and the factors shaping the evolution of sexually dichromatic colouration still remains poorly understood (Shutler and Weatherhead, 1990). Moreover, an overwhelming majority of studies in sexual dichromatism and its evolution have been carried out in vertebrates, particularly in birds (Badyaev and Hill, 2003b; Bortolotti et al., 1996; Cuthill et al., 1999; Eaton, 2005; Gray, 1996; Heinsohn et al., 2005; Martin and Badyaev, 1996), lizards (Cox et al., 2005; LeBas and Marshall, 2000; Macedonia et al., 2002; McCoy et al., 1997) and fish (Chandler and Cabana, 1991; Kodric-Brown, 1998; Skarstein and Folstad, 1996; Ward, 1988). Relatively few studies have investigated sexual dichromatism and its evolution in invertebrates (Miller and Svensson, 2014). Furthermore, most of these studies have dealt with a single or a few species, and comparative studies of sexual dichromatism across multiple closely related species are still scarce. Insects and other arthropods may provide great power to investigate how sexual and natural selection have driven sexually dichromatic colouration and its evolution as they have relatively short generations and can offer potentially large sample size (Eberhard, 2004).

Comparative studies of sexual dichromatism using arthropods such as insects and spiders are probably one of the next most important steps toward understanding the adaptive significance and the evolution of sexual dichromatic colouration.

1.4 Mutual sexual ornamentation

Sexual dimorphic colouration (i.e., sexual dichromatism) is supposed to play an important role in female mate choice, and numerous studies have provided strong evidence for it (Andersson and Simmons, 2006; Cuthill et al., 1999; Gibson and Langen, 1996; Price, 1998b; Taylor and McGraw, 2007; Taylor and McGraw, 2013b). However, it does not mean that sexual monochromatism, where both males and females exhibit similar colours within a species, is not important in mate choice. While males are usually more brightly coloured than females, female showiness is far from uncommon. Conspicuous female colouration occurs in a substantial number of animals (Andersson, 1994; Edward and Chapman, 2011; Kraaijeveld et al., 2007; Prudic et al., 2011). There is evidence that mutual ornamented traits usually exist in socially monogamous species of birds (Moller and Birkhead, 1994), in which conspicuous plumage colouration found in both the sexes is used as an indicator of mate quality. In addition to mate attraction, these mutual conspicuous plumage colours can be an indicator of resource competition out of breeding season (Griggio et al., 2009; Pryke and Griffith, 2007). Tobias et al. (2011) provided evidence to show the mutual ornament song in suboscine birds can help unpaired males and females to advertise for mates as well as for intrasexual resource competition. The mechanisms for the evolution of mutual ornamentations remain mysterious, yet a few studies have suggested that the ornament mutual shared songs in birds are related to the level of

testosterone (Kriner and Schwabl, 1991b; Velando et al., 2001b), immunocompetence, parasite load and egg mass (Martinez-Padilla et al., 2012; Vergara et al., 2011).

Almost all of the previous studies on mutual ornamentation have focused on socially paired birds, but little is known about mutual ornamentation in Arthropoda (Prudic et al., 2011) although it contains overwhelmingly majority of the total number of animal species. This probably because many investigators believe that the ornamentations are mainly for between-pair rather than within-pair interactions (Tobias et al., 2011; West-Eberhard, 1983), and their sexually function is just as a by-product. However, Prudic et al. (2011) showed that reciprocal selection through time may result in mutual sexual ornamentation in a butterfly species *Bicyclus anynana*. Therefore, mutual sexual ornamentations may be more vital and common than we have previously thought in arthropods.

One most acceptable prediction is that the ornament colouration in male is an indicator of male quality (Amundsen, 2000; Hill, 1991a), and females may obtain direct or indirect benefits through colour-based mate-choice. For example, the plumage colouration in male yellowhammer, *Emberiza citronella*, is an indicator of parasite resistance, hence females mated with brighter males can produce more fledglings (Sundberg, 1995). In addition, male exaggerating colouration may also be an indicator of social status in birds (Senar, 1999), fish (Parikh et al., 2006) and mammal (Setchell and Jean Wickings, 2005). However, in most taxa, the association between male ornament colouration and female achieved benefits remain unclear (Johnstone, 1995), and overwhelmingly majority of the studies have focused on sexual dimorphic colourations although there is nothing uncommon for species showing partial sexual dimorphic and partial sexual monomorphic in colours.

1.5 Colour vision in jumping spiders

Jumping spiders, the largest family of spiders (World Spider Catalog, 2015), are diurnal hunting species. Many of them especially males have bright colours and elaborate ornamentation. Jumping spiders are one of the major animal groups in which unique, complex eyes have evolved to support exceptionally high spatial resolution (Land, 1985; Land, 1969b; Land and Nilsson, 2012). Like most of spiders, all salticids have eight simple (i.e. camera-like) eyes arranged into four pairs: a pair of very large anterior median (AM) eyes and, around these, three pairs of smaller secondary eyes (Foelix, 2010) which are different from the AM eyes in both function and morphology (see (Land, 1985) for a review). The secondary eyes are involved in motion detection, but not in the detection of small stationary objects (Duelli, 1978; Land, 1971, 1972). The combined field of view of the secondary eyes encompasses an area nearly 360 º around and above the salticid (Forster, 1977; Jackson and Pollard, 1996; Land, 1969a). This field of view and the sensitivity to movement of these eyes make them the salticid's principal sense organ for prey detection. When moving prey is detected with these eyes, the salticid executes a precise turn that brings the prey into the field of view of its AM eyes (Land, 1971). The AM eyes are primarily responsible for acute vision(Blest et al., 1990; Blest, 1985; Forster, 1985; Land, 1985) and allow salticids to identify objects such as prey, predators, rivals and mates (Jackson and Blest, 1982) and discriminate colours (Jackson, 1977; Nakamura and Yamashita, 2000), including UV colour (De Voe, 1975a; Land, 1969b; Peaslee and Wilson, 1989; Yamashita and Tateda, 1976).

Land (1969), who first examined the retinas of the AM eyes of two jumping spider species, *Phidippus johnsoni* and *Metaphidippus aeneolus*, found that they have

four layers of receptor cells. In his study, he assumed that the layers 1-4 should have red, blue-green, violet-UV, and UV-sensitive visual cells, respectively. DeVoe (1975) documented UV cells (maximum sensitivity at 360 nm), green cells (532 nm) and UV-green cells (both 370 and 525 nm) in the AM eyes of a large salticid *Phiddippus regius*, but he did not find red-sensitive cells by the techniques of intracellular recordings. Blest (1985) later found that *Plexippus validus* was dichromatic, only having two types of photoreceptors: UV (maximum sensitivity at 360 nm) and green (520 nm) cells. Yamashita and Tateda (1976) found that the salticid *Menemerus confuses* from Japan was tetrachromatic: UV (maximum sensitivity at 360 nm), blue (480-500 nm), green (520-540 nm) and yellow cells (580 nm). Their result seems to be consistent with Land's (1969) assumption that each layer of the retina of the AM eyes possesses different photoreceptor cells. The most recently, Peaslee and Wilson (1989) examined the AM eyes of *Maevia inclemens*, and found that this species has an even broader spectral sensitivity extending from UV (330 nm) to deep red (700 nm), with maximum sensitivities in the UV and green regions.

There is considerable disagreement about whether salticids' colour vision is dichromatic, trichromatic or tetrachromatic and about the specific wavelengths of light corresponding to peak sensitivities, but it is widely accepted that salticids in general have UV-sensitive receptors. Controversy concerns primarily sensitivity to long wavelengths. Salticids UV receptors are probably maximally sensitive at 330 – 380 nm (Land, 1969; DeVoe, 1975; Yamashita and Tateda, 1976; Blest et al., 1981; Pearslee and Wilson, 1989). However, knowing that salticids have UV-sensitive receptors does not suffice for showing that UV vision is used for communication during intra-specific interactions. Yet details concerning use of UV vision for intra-specific communication are known for only two salticid species, *Cosmophasis*

umbratica (Lim and Li, 2004, 2006a)and *Phintella vittata* (Li et al., 2008a; Li et al., 2008b). In this study, I extend earlier work by investigating additional salticid species of the subfamily Heliophaninae.

1.6 Sexual dichromatism in jumping spiders

Many salticids appear, to the human eye, to be brightly coloured and to have elaborate ornamentation, with males generally being more conspicuous than females (Oxford and Gillespie, 1998a). The colouration of salticids has often attracted attention, but compared to other colourful invertebrates such as butterflies, salticids are surprisingly poorly studied, and little is known their colouration in intra-specific communication. Studies of animal colouration (Bennett et al., 1994; Cuthill et al., 2000a) have been especially important in illustrating how misleading it can be assume that animals detect the same colours as humans. Therefore, in any study of how an animal uses colour during intra-specific communication, it is essential to use an objective measure of colour.

Several recent studies have indicated that sexual dichromatism may be common in salticids and that sexually dichromatic colouration are also known to be engaged in sexual displays of salticids (Elias et al., 2012; Nelson and Jackson, 2007; Taylor and McGraw, 2007, 2013a; Tedore and Johnsen, 2012b). In addition to human visible colouration (Taylor et al., 2011; Taylor and McGraw, 2007; Taylor and McGraw, 2013b; Tedore and Johnsen, 2012a), UV has also been shown to be crucial in courtship (Li et al., 2008c; Lim et al., 2007a; Lim et al., 2008a) or intrasexal contests (Lim and Li, 2006a, 2013a) in a few species of salticids. These studies indicate that ornament colour patches (including UV colour) usually exist only in males, and that these can increase the male's mating opportunities. It is likely that the

conspicuous colouration of males can be indicator of male's quality (Lim and Li, 2013a; Nelson and Jackson, 2007; Taylor and McGraw, 2007; Taylor and McGraw, 2013b; Tedore and Johnsen, 2012a). However, several limitations to our understanding of the evolution of salticid ornamental colouration still exist, although the role of ornamental colouration in the reproductive behaviour of salticids has been intensively investigated. First, previous studies have usually merely focused on either UV or VIS colours, yet no study has investigated both UV (wavelength: 280-400 nm) and VIS (wavelength: 400-700 nm) colourations together. A recent study (Hu et al., 2012) on the spectral transmission of salticid principal-eye corneas suggested similar potential vision sensitivity. Different colours are probably advertising different aspect of qualities of individuals. For example, Taylor et al. (2014) indicated that the salticid species, *Habronattus pyrrithrix*, showed strong preference for prey with blue colour over prey with red and yellow colour. In this case, red and yellow colours are potentially used by males in order to reduce cannibalism during nuptial dancing. Therefore, analysing colours across both UV and VIS are necessary to get a clear picture about the sexual and ecological roles of colouration in salticids. Second, previous studies have mainly described colours based on qualitative human assessments of colouration simply as blue, red, green etc.. However, each colour can be affected by its total brightness (Qt), which is the total area under the spectral reflectance curve within a specific range of spectral reflectance), chroma (purity or stature of colour) and hue (wavelength at which the spectral reflectance value reaches max in a specific reflectance range). Therefore, it still remains unclear which of these three aspects of colouration play a role in sexual behaviour, and which one is more likely to differ between the two sexes, or among very closely species. Several studies (Li et al., 2008a; Li et al., 2008c; Lim et al., 2007a; Lim and Li, 2006b; Lim et al.,

2008a) have analysed three principal components of UV colour in the salticid species *Cosmophasis umbratica* and *Phintella vittata,* and have successfully evaluated between-sex differences for each principal component. However, these principle components did not show particular meaning of colouration. Third, prior studies have often focused on one species, and no comparative study has been conducted on colouration of multiple species. Investigating sexual dichromatism across multiple species is necessary to get a clear picture of the evolutionary processes behind of colourations in each sex. Furthermore, a comparative study across multiple closely related species enables us to analyse potential correlations between colour differences and speciation events when mapped onto a phylogenetic tree in the future.

1.7 Objectives and thesis structure

Sexual dichromatism is widespread in the animal kingdom and sexual selection are considered to be responsible for its occurrence and evolution. Species that exhibit complex nuptial dancing behaviour usually exhibit exaggerated colouration in males. These ornamented colours are believed to be associated with the high species diversity of that species. Tracing the evolutionary history of ornamental colouration within a clade may inform us whether selection on these colours influences speciation. Therefore, in order to investigate the widespread of sexual dichromatism in jumping spiders, I have investigated sexual dichromatism across multiple species of jumping spider from the genus *Phintella* by analysing spectral reflectance across UV (280-400 nm) and human visible (400-700 nm) wavelength ranges in **Chapter 2**. After the investigation of sexual dichromatism, I then tested the importance of sexual dimorphic UV in mate-choice in eight species of *Phintella* in **Chapter 3**. Colour-based mate-choice is common in animal kingdom, but overwhelmingly majority of

the studies are focusing on conspicuous sexually dimorphic colours. It is not uncommon, however, for both sexes of a species to show similar ornamental colouration (usually partial sexual dichromatism and partial sexual monochromatism) in nature. *Chrysilla acerosa* is such a salticid species in which both males and females exhibit, at least to human perception, the similar ornament colours in some body regions. In **Chapter 4,** I compared between-sex colour variation (including UV) in *C. acerosa* and then examined the correlation between male colouration and mating success in this species. The results from **Chapter 4** showed that *C. acerosa* females used sexually monomorphic (SM) colour to make mate-choice decision although sexually dimorphic (SD) colouration also existed in this species. Hence I have investigated whether females obtained direct or indirect benefits based on either sexually monomorphic or dimorphic colour following the last section of study in this chapter. To elucidate the origin and evolution of colouration in the subfamily Heliophaninae with focus on the genus *Phintella*, in **Chapter 5**, I first reconstructed the phylogenetic tree of Heliophaninae using five genes and then traced the origin and evolution of sexual colour dimorphism by mapping the presence of sexual dichromatism, the total brightness (Qt), hue and chroma separately to the tree for both UV and VIS reflectance ranges.

CHAPTER 2

Sexual dimorphism in colouration of nine jumping spider species of the genus *Phintella* (Araneae: Salticidae)

2.1 Introduction

Sexual dimorphism is a phenotypic difference between males and females within the same species. It is widespread in the animal kingdom and usually evolves when a particular trait is subjected to sexual selection (Campbell, 1972). Numerous studies have shown that sexual selection contributes to sexual dimorphism in a wide range of species (Andersson, 1994). Furthermore, some studies imply that sexually dimorphic traits are often related to speciation events (Gage et al., 2002a; Kraaijeveld et al., 2011; Ritchie, 2007b; Runemark and Svensson, 2012; Singh and Singh, 2014). On the other hand, several recent studies have shown that ecological factors are also crucial for the development of sexually dimorphic characters (Gorman et al., 2014; Oneal and Knowles, 2013; Sousa and Westneat, 2013). Therefore, sexually dimorphic traits can be primarily driven by sexual selection, or reflect the influence of both sexual and natural selection (Miller and Svensson, 2014). It is still not clearly understood which aspects of a particular sexual dimorphic trait are driven by sexual selection (Hunt et al., 2009) and which aspects are driven by natural selection (Ingleby et al., 2010).

'Sexual dichromatism' refers to inter-sex variation in colouration within a species, and it can be driven by both sexual and natural selection (Ibáñez et al., 2013). Conspicuous colour patterns in animal body surfaces often play a vital role in female mate choice, male-male competition o both (reviewed in Andersson 1994). There is evidence that, in certain cases, ecological factors are considered to be the driving force of conspicuous colouration differences between the two sexes (Ciccotto et al., 2014).

In a review of birds, Rajchard (2009) described dimorphic colour plumage as an important visual cue for sex recognition in strong sexually dimorphic species.

Another evidence proposed that, even with slight sexual plumage colour differences, for example in the bird species, females may use colour to recognize different males from different geographic populations, and they prefer to mate with males from the same geographic population with themselves (Sœtre, 1993). The evolutionary process of ornamental colouration in some birds can most probably be interpreted as the correlation between a male's quality and its plumage brightness (Hill, 1991b). In his study, Hill (1991b) pointed out that male plumage colouration is correlated with nest attractiveness and overwintering survival. Additionally, there is a positive correlation between plumage brightness of fathers and sons. A number of similar studies have also been conducted in fishes. For example, females from two species of the genus *Undamilia* are known to select mates based on the male's nuptial colouration. Furthermore, a sexual selection experiment indicated that females preferred males with extreme colours rather than males with intermediate colours (Stelkens et al., 2008). One recent research suggested that female mating preference and male ornamental colouration may have coevolved in a lake Victoria cichlid fish (Maan et al., 2010). Brighter colouration in male fish can be a reliable indicator of high quality, which enables males to own larger territories and lower parasite infestation rates (Maan et al., 2006). Interestingly, ornamental colouration has also been used by females to attract males. For example, in a fish species, males prefer to mate with females that have the same colour morph as their mother (Pierotti et al., 2008).

Compared with the sexual function of ornamental colouration in males, their ecological roles are relatively unstudied. Therefore, it is not surprising that the mechanisms whereby ecological factors drive the evolution of ornamental colour patterns are less clear compared with the sexual selection. There are several different explanations as to how ecological factors can drive sexual dichromatism. According a

study of birds (Badyaev and Hill, 2003b), two ecological factors are crucial for driving the evolution of sexual dichromatism: predation pressure from the predators around the nest and the specific habitat driving force. Sexual colouration differences may be the result of predator selection for crypsis in females. Therefore, it is not the males that gain ornamental colourations but females lost the conspicuous colouration over evolutionary time. Another possibility is that the plumage ornamentations in birds are often changing when birds occupied new habitats (Badyaev and Hill, 2003b). Another study (Cooper, 2010) provided the evidence supporting the second point, in which the inter-sex variation in colour pattern in a damselfly species *Megalagrion calliphya* is most probably driven by natural UV light.

These studies have attempted to get a clear picture about the association between sexual dichromatic colouration and sexual selection as well as natural selection. However, it remains unclear about the connections between a specific sexual dichromatic colouration and factors, what is cause and what is effect in the relationship (Shutler and Weatherhead, 1990). On the other hand, an overwhelming majority of studies on sexual dichromatism were carried out on birds, lizards and fishes. Repeat studies in number and experimental trials are relatively limited (Miller and Svensson, 2014). Therefore, more studies are needed in invertebrates such as insects and spiders to provide evidence on the evolutionary mechanisms of sexual dichromatic colouration. Insects and spiders may provide great power to investigate natural and sexual selection on sexual dichromatism as they have relatively short generations and can offer potentially large sample sizes (Miller and Svensson, 2014). Nevertheless, comparative studies of sexual dichromatism among multiple species are probably one of the next most important steps toward understanding the adaptive significance and the evolution of sexual dichromatic colouration in insects and spiders.

Hence in order to investigate the evolutionary history of the ornamental sexual dichromatic colouration, I conducted a comparative study of sexual dichromatism using nine spices of jumping spider (family Salticidae) from the genus *Phintella* from Asia. Salticidae is the largest family of spiders with 586 genera and 5813 recorded species (World Spider Catalog, 2015). Based on our field collections and observations, at least to a human observer, many salticid species exhibit obvious sexual dimorphism in colouration. In addition to high species diversity and ubiquity of sexual dichromatic colouration, salticids have sophisticated visual systems (including UV vision). The principal eyes of the salticid species *Phidippus reqius* contain three types of photoreceptors, UV cells, UV-Green sensitive cells and green sensitive cells (De Voe, 1975a). Moreover, the salticid species *Hasarius asansoni* can discriminate blue, green, yellow and red papers (Nakamura and Yamashita, 2000). Another study (VanderSal and Hebets, 2007) has even shown that salticids can learn to discriminate colours.

Several recent studies have indicated that ornamental colouration in salticid males are usually engaged in sexual display showing behaviour (Elias et al., 2012; Nelson and Jackson, 2007; Taylor and McGraw, 2007, 2013a; Tedore and Johnsen, 2012b). In addition to human visible colouration, UV has also been shown to be crucial in courtship (Li et al., 2008c; Lim et al., 2007a; Lim et al., 2008a) or intra-sexual contests (Lim and Li, 2006a, 2013a) in some salticid species. These studies indicate that ornamental colour patches (including UV colour) usually exist only in males, and that these can increase the male's mating opportunities. It is likely that the conspicuous colouration on males can be indicator of male's quality (Lim and Li, 2013a). Oxford and Gillespie (1998a) suggested that sexual dimorphic traits are probably responsible for the high species diversity of salticids. These characters are commonly connected to sexual behaviour which is considered to be crucial for

speciation events in highly sexually dimorphic animals. As mentioned previously, in addition to their sexual function, ornamental colour patterns in salticids are also probably involved in ecological interactions (Oxford and Gillespie, 1998a).

Several limitations to our understanding of the evolution of salticid ornamental colouration still exist, although the role of ornamental colouration in the reproductive behaviour of salticids has been intensively investigated. First, previous studies have usually merely focused on either UV or VIS colours, yet no study has investigated both UV (wavelength: 280-400 nm) and VIS (wavelength: 400-700 nm) colourations. A recent study (Hu et al., 2012) on the spectral transmission of salticid principal-eye corneas suggested similar potential vision sensitivity. Different colour probably are the different indicators to spiders. For example, one study (Taylor et al., 2014) indicated that the salticid species, *Habronattus pyrrithrix*, showed strong preference for prey with blue colour over prey with red and yellow colour. In this case, red and yellow colours are potentially used by males in order to reduce cannibalism during nuptial dancing. Therefore, analysing colours across both UV and VIS are necessary to get a clear picture about the sexual and ecological roles of colouration in salticids. Second, previous studies have mainly described colours based on qualitative human assessments of colouration simply as blue, red, green etc.. However, each colour can be affected by its total brightness (Qt), which is the total area under the spectral reflectance curve within a specific range of spectral reflectance), chroma (purity or stature of colour) and hue (wavelength at which the spectral reflectance value reaches max in a specific reflectance range). Therefore, it still remains unclear which of these three aspects of colouration play a role in sexual behaviour, and which one is more likely to differ between the two sexes, or among very closely species. Several studies (Li et al., 2008a; Li et al., 2008c; Lim et al., 2007a; Lim and Li, 2006b; Lim et al.,

2008a) have analysed three principal components of UV colour in the salticid species *Cosmophasis umbratica* and *Phintella vittata*, and have successfully evaluated between-sex differences for each principal component. However, these principle components did not show particular meaning of colouration. Third, prior studies have often focused on one species, and no comparative study has been conducted on colouration of multiple species. Here I investigated inter- and intraspecific colouration variations of nine salticid species from the genus *Phintella* from Asia. Investigating sexual dichromatism across multiple species is necessary to get a clear picture of the evolutionary processes behind of colourations in each sex. Furthermore, a comparative study across multiple closely related species enables us to analyse potential correlations between colour differences and speciation events when mapped onto a phylogenetic tree in the future.

2.2 Material and methods

2.2.1 Study subjects and maintenance

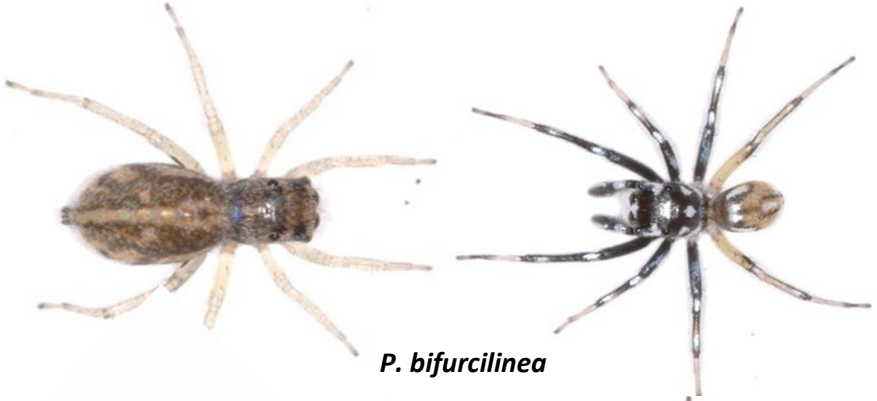
Phintella species are widely distributed in tropical, sub-tropical and temperate area (Platnick, 2014). The nine *Phintella* species (**Fig. 2.1**) were collected from different countries and regions of Asia (**Table 2.1**). They were collected as either adults or large juveniles by beating shrubs, and then transported to the laboratory at the National University of Singapore (Singapore). They were kept individually in plastic cylindrical cages (diameter × height: 60 mm × 80 mm) and maintained in a laboratory with controlled environmental conditions (relative humidity: 80-90%; temperature: 25 ± 1 ℃; light regim: 12:12 h light/dark cycle; lights on 08:00 h) followed a standard protocol used in early salticid studies (Lim & Li., 2004; 2006a, b; Li et al., 2008a, b). Fruit flies (*Drosophila melanogaster*) were provided to them twice a week as food.

Table 2.1. Collection locality details of the nine *Phintella* species in this investigation.

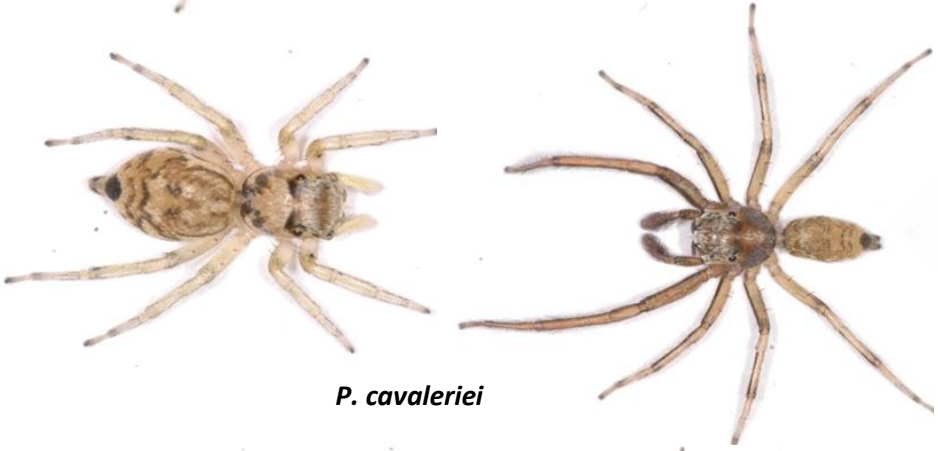
Species	Collected location	Dimension (N)/ Longitude(E)/ Altitude (m)
Phintella arenicolor	Changsha, Hunan, China	28°15'/ 112°14' / 118
P. bifurcilinea	XTBG, Yunnan, China	21°56'/ 101°16'/ 571
P. cavaleriei	Erlangping, Xixia county, Nanyang, Henan, China	33°32' / 111°41' / 501
P. linea	Jiugongshan, Tongshan county, Xianning, Hubei, China	29°25' / 114°40' / 1129
P. vittata	XTBG, Yunnan Province, China	21°54' / 101°15' / 559
***Phintella* sp.4**	Xishuangbanna Tropical Botanic Garden (XTBG), Yunnan ,China	21°56' / 101°15' / 565
***Phintella* sp.6**	Zhongliao,Nantou county, Taiwan	30°54' / 120°47' / 213
***Phintella* sp.8**	XTBG, Yunnan, China	21°53' / 101°14' / 577
***Phintella* sp.14**	Northern University of Malaysia (UUM) Sintok, Kedah, Malaysia	6°28' / 100°30' / 277

Female **Male** **Female** **Male**

Phintella arenicolor



P. bifurcilinea



P. cavaleriei

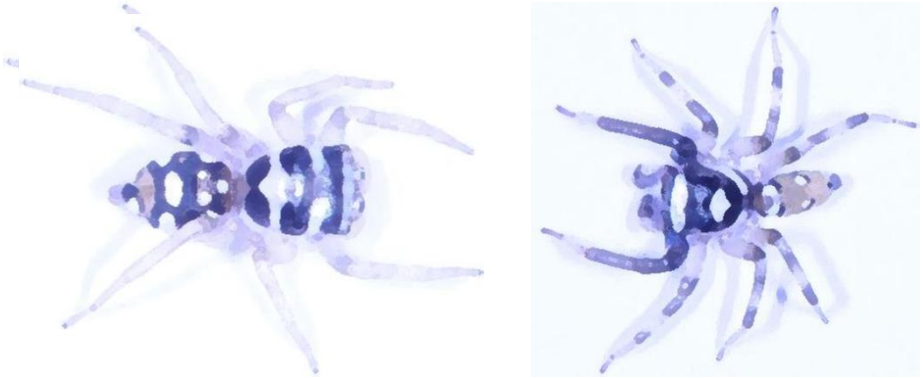
P. linea

P. vittata

***Phintella* sp.4**

***Phintella* sp.6**

***Phintella* sp.8**



***Phintella* sp.14**

Figure 2.1. Male and female of the nine species of *Phintella* used in this study.

2.2.2 Spectrophotometeric measurements

The spectral reflectance of adult spiders was measured within three days after being transported to the laboratory. For the juveniles, spectral reflectance was measured approximately two weeks after they had moulted to maturity. I measured the reflectance spectra of all the nine species for both the sexes following the standard protocols as used in previous studies (Cuthill et al., 1999; Endler and Thery, 1996; Lim and Li, 2006b). Four body regions were selected for spectral reflectance measurements based on nuptial display behaviour as described in previous salticid studies (Li et al., 2008a; Li et al., 2008c; Lim and Li, 2006b). The four body regions are: (1) dorsal abdomen, (2) dorsal carapace, (3) lateral abdomen, and (4) lateral carapace. To collect the spectra reflectance data, I used an Ocean Optics USB4000 spectrometer (Ocean Optics Inc.) and a DH2000 deuterium & tungsten halogen light source (Ocean Optics Inc., USA). Each individual was anaesthetized with CO_2 and then mounted on a fixed stage for spectra reflectance measuring. The reflectance probe was kept consistently at 2 mm from the spider body and at a right angle from the measurement surface. Details of the method were consistent with a previous study (Lim and Li, 2006b). Five reflectance spectra were measured from each specific body region for each individual. These spanned ultraviolet (UV: < 400 nm) and human visual wavelength (VIS: 400–700 nm) ranges. I collected a total number of 1440 reflectance spectra from these nine *Phintella* species (5 individuals for each sex of each species).

2.2.3 Data analysis

Spectra were taken at 10 nm intervals for each of the original reflectance spectrum (spectra range = 300-700 nm). I used AVICOL V.6 (GOMEZ, 2006) to analyse the

spectral reflectance data, and three colour components were analysed for each ranges (UV and VIS): total brightness (the total area under a particular line within a specific range of spectral reflectance), hue (the wavelength at which the reflectance is maximal in the particular spectra reflectance range), and maxmin chroma (maxmin chroma = abs ((Rmax - Rmin) / Rav) with Rav =λλ)) (chroma hereafter). All the spectral data were normalised (see AVICOL v.6 chapter 8 (GOMEZ, 2006) for more details).

First of all, I performed general liner model (GLM) repeated-measures multivariate analysis of variance (MANOVA) to tests the overall effects of species, body region and sex on colour components (total brightness, hue and chroma of both the UV and VIS wavelength ranges) of the nine species. I then conducted separate MANOVAs for UV and VIS ranges if there were overall significance effects found from GLM. Finally, I used one-way ANOVA to test the effects of species, body region and sex on the total brightness, hue and chroma of UV and VIS range, separately. All the analyses were performed using SPSS, version 16.0.

2.3 Results

2.3.1 Interspecific variation in colouration

2.3.1.1 Overall variation

Results from MANOVA showed a significant overall effect of species (Wilk's λ = 0.736, $F_{8, 8}$ = 17, P < 0.001) and body region (Wilk's λ = 0.878, $F_{3, 3}$ = 7.7, P < 0.001) on full-spectral colouration (UV+VIS), but no significant effect of sex on the colouration of nine species was found (Wilk's λ = 0.933, $F_{1, 1}$ = 0.1, P = 0.784). When the UV and VIS reflectance ranges were analysed separately, there were significant variation among species (UV: Wilk's λ = 0.316, $F_{8, 16}$ = 39.7, P < 0.001; VIS: Wilk's λ = 0.812, $F_{8, 16}$ = 11, P < 0.001), among body regions (UV: Wilk's λ = 0.954, $F_{3, 6}$ = 2.8, P = 0.04, Wilk's λ = 0.812, $F_{3, 6}$ = 14.3, P < 0.001), and between the sexes (UV: Wilk's λ = 0.904, $F_{1, 2}$ = 32.3, P < 0.001; VIS: Wilk's λ = 0.969, $F_{1, 2}$ = 8.9, P = 0.003) for both UV and VIS wavelength ranges.

2.3.1.2 Variation in three colour components

Results from MANOVAs revealed significant differences in the total brightness and hue in UV range, but there was no significant variation in UV chroma among species, among body region and between the sexes (**Table 2.2:** UV). In VIS range, there were significant differences in all three colour components (Qt, hue and chroma) among species, among body regions and between the sexes except that no significant difference in total brightness was found between the sexes (**Table 2.2:** VIS).

Table 2.2. The nine *Phintella* species colouration differences (by three colour components) between species, between body regions and between sexes. UV+VIS: Wavelength 300-700 nm; UV: Wavelength 300-400 nm; VIS: Wavelength 400-700 nm.

Wavelength range	**Colour matric**		**Wilks'**	**F**	***P***
		Species	0.91	$F_{8, 32} = 28.5$	<0.001
	Qt	Body region	0.95	$F_{3, 12} = 2.9$	0.036
		Sex	1.00	$F_{1, 4} = 10.1$	0.002
		Species	0.90	$F_{8, 32} = 43.1$	<0.001
UV	Hue	Body region	0.96	$F_{3, 12} = 2.7$	0.048
		Sex	0.98	$F_{1, 4} = 35.4$	<0.001
		Species	0.92	$F_{8, 32} = 29.6$	<0.001
	Chroma	Body region	0.95	$F_{3, 12} = 1.7$	0.171
		Sex	0.98	$F_{1, 4} = 0.4$	0.514
		Species	0.92	$F_{8, 32} = 21.2$	<0.001
	Qt	Body region	0.96	$F_{3, 12} = 5.2$	0.001
		Sex	0.99	$F_{1, 4} = 0.7$	0.389
		Species	0.89	$F_{8, 32} = 11.8$	<0.001
VIS	Hue	Body region	0.97	$F_{3, 12} = 18.6$	<0.001
		Sex	0.99	$F_{1, 4} = 11$	0.001
		Species	0.93	$F_{8, 32} = 58$	<0.001
	Chroma	Body region	0.97	$F_{3, 12} = 20.9$	<0.001
		Sex	0.99	$F_{1, 4} = 4.8$	0.029

2.3.1.3 Interspecific colour variation

When both UV and VIS were taken into consideration in a combination, the three species *P. arennicolor, P. vittata* and *Phintella* sp. 14 did not show any significant difference in colouration to each other, but all indicated notable reflectance variations to all the rest six species. However, there was no significant difference in spectral reflectance happened among the six species. In other words, the nine *Phintella* species could be divided into two groups based on the combined colour reflectance of UV and VIS, the first group contains species of *P. arennicolor, P. vittata* and *Phintella* sp. 14, and the second group includes the rest six species in study (**Table 2.3**: UV+VIS). However, the UV reflectance variations were different from VIS reflectance variations when they were analysed separately, and more species showed considerable interspecies reflectance difference in UV range than in VIS range (**Table 2.3**: UV and VIS). One notable species is *P. bifurcilinea*, which showed no significant difference in VIS range from the rest species except for *P. arenicolor,* but it indicated significant reflectance variation in UV range to other five species. The similar patterns also happened in *Phintella* sp. 4 (**Table 2.3**: UV and VIS).

Table 2.4 presents the details of interspecific colour variation based on colour components (Qt, hue and chroma) in the UV and VIS range, respectively. In general, Qt and hue showed more notable interspecific variation than chroma in the UV range. *Phintella* sp. 8 even showed significant differences in the Qt and hue in the UV from all the other eight species. However, although *P. vittata* showed a significant difference in the UV-chroma from all the other eight species, there were no significant interspecies differences in the UV-chroma among the rest eight species except that there was a significance difference in the UV-chroma between *Phintella* sp. 8 and *P. bifurcilinea*. In VIS reflectance range, both *P. arenicolor* and *P. vittata* showed

significant differences in the Qt from the other seven species, but there was no significant difference in the Qt in between these two species. Unlike in the Qt, there were significant between-species differences in the VIS hue. In the VIS chroma, *P. vittata* showed significant differences from all the other species, and *P. cavaleriei* showed significant difference to other seven species except for *P. bifurcilinea*.

Table 2.3. A matrix showing among-species colour reflectance overall variation (MANOVA) for the full reflectance spectrum (UV+VIS), UV and VIS wavelength range. *P*-value (post-hoc paired comparisons) is reported.

Wavelength range		***P. arenicolor***	***P. bifurcilinea***	***P. cavaleriei***	***P. linea***	***P. vittata***	***Phintella* sp.4**	***Phintella* sp.6**	***Phintella* sp.8**	***Phintella* sp. 14**
	P. arenicolor									
	P. bifurcilinea	<0.001								
	P. cavaleriei	<0.001	0.867							
	P. linea	<0.001	1	0.993						
UV+VIS	*P. vittata*	0.998	<0.001	<0.001	<0.001					
	Phintella sp.4	<0.001	1	0.779	0.998	<0.001				
	Phintella sp.6	0.001	0.983	0.235	0.799	<0.001	0.995			
	Phintella sp.8	<0.001	1	0.994	1	<0.001	0.997	0.788		
	Phintella sp.14	0.998	<0.001	<0.001	<0.001	0.858	<0.001	0.011	<0.001	
	P. arenicolor									
	P. bifurcilinea	<0.001								
	P. cavaleriei	0.836	0.069							
	P. linea	0.188	0.585	0.978						
UV	*P. vittata*	0.004	<0.001	<0.001	<0.001					
	Phintella sp.4	<0.001	0.998	0.006	0.156	<0.001				
	Phintella sp.6	0.716	<0.001	0.03	0.001	0.469	<0.001			
	Phintella sp.8	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
	Phintella sp.14	0.996	<0.001	0.309	0.019	0.064	<0.001	0.991	<0.001	
	P. arenicolor									

VIS	*P. bifurcilinea*	0.009							
	P. cavaleriei	<0.001	0.069						
	P. linea	<0.001	0.833	0.872					
	P. vittata	0.983	0.186	<0.001	0.001				
	Phintella sp.4	0.053	1	0.013	0.486	0.494			
	Phintella sp.6	<0.001	0.631	0.969	1	<0.001	0.28		
	Phintella sp.8	0.945	0.29	<0.001	0.003	1	0.644	0.001	
	Phintella sp.14	0.956	0.265	<0.001	0.003	1	0.612	0.001	1

Table 2.4. A matrix showing the comparisons among-species colour reflectance variation by colour components for both the UV and VIS wavelength ranges *P*-value (post-hoc paired comparisons) is reported.

Wavelength range	**Colour matric**		***P. arenicolor***	***P. bifurcilinea***	***P. cavaleriei***	***P. linea***	***P. vittata***	***Phintella* sp.4**	***Phintella* sp.6**	***Phintella* sp.8**	***Phintella* sp.14**
		P. arenicolor									
		P. bifurcilinea	0.002								
		P. cavaleriei	1	0.001							
		P. linea	0.001	1	<0.001						
	Qt	*P. vittata*	0.133	<0.001	0.201	<0.001					
		Phintella sp.4	<0.001	0.906	<0.001	0.966	<0.001				
		Phintella sp.6	0.02	0.999	0.011	0.995	<0.001	0.54			
		Phintella sp.8	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
		Phintella sp.14	<0.001	0.95	<0.001	0.986	<0.001	1	0.642	<0.001	
		P. arenicolor									
UV		*P. bifurcilinea*	0.014								
		P. cavaleriei	0.261	0.978							
		P. linea	1	0.053	0.523						
	Hue	*P. vittata*	0.01	<0.001	<0.001	0.002					
		Phintella sp.4	0.014	1	0.978	0.053	<0.001				
		Phintella sp.6	<0.001	<0.001	<0.001	<0.001	0.672	<0.001			
		Phintella sp.8	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
		Phintella sp.14	<0.001	<0.001	<0.001	<0.001	0.578	<0.001	1	<0.001	
		P. arenicolor									
	Chroma	*P. bifurcilinea*	0.999								
		P. cavaleriei	0.75	0.98							

		P. linea	1	0.999	0.702				
		P. vittata	<0.001	<0.001	<0.001	<0.001			
		Phintella sp.4	1	0.966	0.403	1	<0.001		
		Phintella sp.6	0.972	1	1	0.958	<0.001	0.789	
		Phintella sp.8	0.981	<0.001	0.999	0.97	<0.001	0.824	1
		Phintella sp.14	0.296	0.718	0.999	0.255	<0.001	0.093	0.939	0.92
		P. arenicolor							
		P. bifurcilinea	<0.001						
		P. cavaleriei	<0.001	0.49					
		P. linea	<0.001	1	0.196				
	Qt	*P. vittata*	1	<0.001	<0.001				
		Phintella sp.4	<0.001	1	0.736	0.994	<0.001		
		Phintella sp.6	<0.001	0.999	0.91	0.952	<0.001	1	
		Phintella sp.8	<0.001	0.998	0.109	1	<0.001	0.971	0.87
		Phintella sp.14	<0.001	0.358	1	0.123	<0.001	0.603	0.823	0.064
VIS		*P. arenicolor*							
		P. bifurcilinea	0.955						
		P. cavaleriei	<0.001	<0.001					
		P. linea	0.093	0.752	0.085				
	Hue	*P. vittata*	0.934	1	<0.001	0.801			
		Phintella sp.4	0.999	1	<0.001	0.411	1		
		Phintella sp.6	0.005	0.179	0.55	0.991	0.215	0.048	
		Phintella sp.8	0.484	0.03	<0.001	<0.001	0.023	0.123	<0.001	
		Phintella sp.14	0.996	0.528	<0.001	0.007	0.469	0.847	<0.001	0.939
	Chroma	*P. arenicolor*							

P. bifurcilinea	0.005							
P. cavaleriei	0.302	<0.001						
P. linea	0.004	1	<0.001					
P. vittata	<0.001	<0.001	<0.001	<0.001				
Phintella sp.4	0.001	1	<0.001	1	<0.001			
Phintella sp.6	0.738	0.469	0.002	0.437	<0.001	0.233		
Phintella sp.8	<0.001	0.893	<0.001	0.91	<0.001	0.984	0.014	
Phintella sp.14	0.072	0.996	<0.001	0.994	<0.001	0.95	0.937	0.382

2.3.2 Colour variation within species

2.3.2.1 Colour variation by colour ranges

Table 2.5 shows between sex and between body region variations in the full spectral range (UV+VIS), as well as UV and VIS reflectance ranges for each of the nine species. In general, three species (*Phintella linea*, *Phintella* sp. 6 and *Phintella* sp. 14) and five species (*Phintella arenicolor*, *P. cavaleriei*, *Phintella* sp. 4, *Phintella* sp. 6 and *Phintella* sp. 8) showed significant between-sex and between body region differences in the full spectral range (wavelength: 300-700 nm), respectively. In the UV range, six species (*Phintella arenicolor, P. bifurcilinea, P. linea Phintella* sp.4 *Phintella* sp.8, *Phintella* sp.) exhibited significant intersex variations, and all nine species except for *P. arenicolor* and *P. vittata* showed significant inter-body region variations, respectively. However, in the VIS reflectance range, only four species and five species showed significant between-sex and between-body region variations, respectively. All of the nine species showed significant between-body region variations in both UV and VIS reflectance ranges expect that *P. arenicolor* showed significant between-body region variations only in VIS range **(Table 2.6).**

Table 2.5. Results from ANOVAs testing between-sex and between-body region colouration differences in each of the nine *Phintella* species. UV+VIS: wavelength range 300-700 nm; UV: colour range: 300-400 nm; VIS: colour range 400-700 nm.

Species		Between sex			Between body region		
		UV+VIS	UV	VIS	UV+VIS	UV	VIS
Phintella arenicolor	Wilks'	0.99	0.59	0.98	0.59	0.79	0.55
	F	$F_{1, 1} = 0.4$	$F_{1, 2} = 9.5$	$F_{1, 2} = 0$	$F_{3, 3} = 8.1$	$F_{3, 6} = 0.8$	$F_{3, 6} = 8.3$
	P	0.539	0.004	0.95	<0.001	0.522	<0.001
P. bifurcilinea	Wilks'	0.72	0.49	0.63	0.75	0.58	0.52
	F	$F_{1, 1} = 0.5$	$F_{1, 2} = 19.4$	$F_{1, 2} = 5.9$	$F_{3, 3} = 1.6$	$F_{3, 6} = 3.1$	$F_{3, 6} = 2.8$
	P	0.494	<0.001	0.021	0.199	0.037	0.056
P. cavaleriei	Wilks'	1	0.78	0.82	0.69	0.39	0.5
	F	$F_{1, 1} = 2.1$	$F_{1, 2} = 0.9$	$F_{1, 2} = 0.9$	$F_{3, 3} = 10.4$	$F_{3, 6} = 12.3$	$F_{3, 6} = 3.8$
	P	0.157	0.35	0.352	<0.001	<0.001	0.018
P. linea	Wilks'	0.81	0.36	0.79	0.79	0.3	0.57
	F	$F_{1, 1} = 5$	$F_{1, 2} = 60.7$	$F_{1, 2} = 0.5$	$F_{3, 3} = 0.1$	$F_{3, 6} = 6.9$	$F_{3, 6} = 1.5$
	P	0.032	<0.001	0.494	0.947	0.001	0.241
P. vittata	Wilks'	0.85	0.85	0.69	0.57	0.78	0.47
	F	$F_{1, 1} = 0.9$	$F_{1, 2} = 0.5$	$F_{1, 2} = 3.4$	$F_{3, 3} = 1.5$	$F_{3, 6} = 0.8$	$F_{3, 6} = 5.1$
	P	0.346	0.475	0.075	0.243	0.512	0.005
***Phintella* sp.4**	Wilks'	0.82	0.8	0.89	0.54	0.46	0.51
	F	$F_{1,1} = 0.8$	$F_{1, 2} = 8.8$	$F_{1, 2} = 4.2$	$F_{3, 3} = 8.7$	$F_{3, 6} = 4.5$	$F_{3, 6} = 10$
	P	0.368	0.005	0.049	<0.001	0.009	<0.001
***Phintella* sp.6**	Wilks'	0.25	0.94	0.24	0.12	0.33	0.13
	F	$F_{1, 1} = 55.4$	$F_{1, 2} = 1.8$	$F_{1, 2} = 98.8$	$F_{2, 3} = 29.5$	$F_{3, 6} = 12.4$	$F_{3, 6} = 67$
	P	<0.001	0.187	<0.001	<0.001	<0.001	<0.001
***Phintella* sp.8**	Wilks'	0.85	0.71	0.69	0.75	0.46	0.89
	F	$F_{1, 1} = 2.1$	$F_{1, 2} = 8.2$	$F_{1, 2} = 1.4$	$F_{3, 3} = 7.9$	$F_{3, 6} = 10.2$	$F_{3, 6} = 0.3$
	P	0.156	0.007	0.248	<0.001	<0.001	0.812
***Phintella* sp.14**	Wilks'	0.53	0.59	0.57	0.95	0.61	0.12
	F	$F_{1, 1} = 20.8$	$F_{1, 2} = 18.4$	$F_{1, 2} = 26.3$	$F_{3, 3} = 0.1$	$F_{3, 6} = 4$	$F_{3, 6} = 0.3$
	P	<0.001	<0.001	<0.001	0.939	0.015	0.826

Table 2.6. Results from ANOVAs testing between-body region differences at each colour component (Qt, Hue and Chroma) for each of the nine *Phintella* species.

Species		UV			VIS		
		Qt	**Hue**	**Chroma**	**Qt**	**Hue**	**Chroma**
P. arenicolor	Wilk's λ	0.74	0.74	0.59	0.75	0.83	0.55
	$F_{3, 12}$	2.5	0.5	0.6	7.2	8.3	3.2
	P	0.072	0.676	0.61	0.001	<0.001	0.037
P. bifurcilinea	Wilk's λ	0.66	0.78	0.74	0.56	0.7	0.78
	$F_{3, 12}$	7.9	0.5	0.5	6.3	3.1	11
	P	<0.001	0.698	0.718	0.002	0.039	<0.001
P. cavaleriei	Wilk's λ	0.76	0.57	0.68	0.57	0.74	0.65
	$F_{3, 12}$	5.4	10.3	5.7	5	3.1	9.2
	P	0.004	<0.001	0.003	0.006	0.038	<0.001
P. linea	Wilk's λ	0.46	0.53	0.68	0.61	0.56	0.61
	$F_{3, 12}$	23.2	1	2.6	8.5	2.4	19.7
	P	<0.001	0.384	0.071	<0.001	0.082	<0.001
P. vittata	Wilk's λ	0.68	0.7	0.6	0.59	0.58	0.68
	$F_{3, 12}$	0.6	1.5	3.2	1.2	9.4	1.8
	P	0.6	0.233	0.037	0.34	<0.001	0.172
***Phintella* sp.4**	Wilk's λ	0.25	0.65	0.7	0.73	0.67	0.68
	$F_{3, 12}$	12.5	4777	1.2	4.8	8.6	157.4
	P	<0.001	<0.001	0.341	0.006	<0.001	<0.001
***Phintella* sp.6**	Wilk's λ	0.46	0.41	0.2	0.08	0.3	0.48
	$F_{3, 12}$	8.6	4.2	6.9	30	75.2	5.8
	P	<0.001	0.012	0.001	<0.001	<0.001	0.003
***Phintella* sp.8**	Wilk's λ	0.75	0.72	0.53	0.73	0.66	0.74
	$F_{3, 12}$	10.5	7.3	2.4	1.1	0.4	1.2
	P	<0.001	0.001	0.085	0.379	0.788	0.32
***Phintella* sp.14**	Wilk's λ	0.39	0.83	0.57	0.58	0.66	0.76
	$F_{3, 12}$	3.6	2.3	4	0.6	0.4	3.8
	P	0.024	0.095	0.015	0.594	0.731	0.018

2.3.2.2 Intersex variation by colour components

Only *P. bifurcilinea* showed significant intersex differences in all of the three colour components (Qt, hue and chroma) of UV reflectance, whereas other five species exhibited a significant intersex difference either in the UV Qt (four species) or in the UV hue (one species) (**Table 2.7**: UV). In the VIS range, although no species showed significant intersex differences in all the three colour components, six species showed a significant intersex variation in two of the three colour components, and the other three species exhibited significant intersex variation either in the hue or chroma **(Table 2.7:** VIS).

Table 2.7. Results from ANOVAs testing between-sex difference at each colour component (Qt, Hue and Chroma) for each of the nine *Phintella* species.

Species		UV			VIS		
		Qt	**Hue**	**Chroma**	**Qt**	**Hue**	**Chroma**
P. arenicolor	Wilk's λ	0.87	0.74	0.9	0.85	0.97	0.79
	$F_{1, 4}$	22.6	2.6	2.7	0.1	0	5.7
	P	<0.001	0.114	0.108	0.768	0.918	0.023
P. bifurcilinea	Wilk's λ	0.82	0.79	0.92	0.7	0.88	0.85
	$F_{1, 4}$	4.8	32.7	7.2	13	9.6	2.2
	P	0.035	<0.001	0.011	0.001	0.004	0.146
P. cavaleriei	Wilk's λ	0.95	0.95	0.74	0.71	0.83	0.8
	$F_{1, 4}$	10.1	0	0.7	5.1	0	6.7
	P	0.003	0.968	0.417	0.031	0.981	0.014
P. linea	Wilk's λ	0.86	0.87	0.97	0.94	0.88	0.92
	$F_{1, 4}$	1.3	55.4	0	9.1	1.2	9.6
	P	0.26	<0.001	0.98	0.005	0.288	0.004
P. vittata	Wilk's λ	0.76	0.97	0.8	0.76	0.9	0.91
	$F_{1, 4}$	4.1	0	2.9	2.7	9.1	3.7
	P	0.051	0.94	0.095	0.108	0.005	0.062
***Phintella* sp.4**	Wilk's λ	0.83	0.72	0.86	0.92	0.88	0.9
	$F_{1, 4}$	1.3	1.1	4	0.6	4.2	2.9
	P	0.269	0.351	0.053	0.459	0.048	0.05
***Phintella* sp.6**	Wilk's λ	0.66	0.68	0.72	0.13	0.69	0.88
	$F_{1, 4}$	2.1	1.2	2.8	46	112	0.5
	P	0.159	0.285	0.101	<0.001	<0.001	0.477
***Phintella* sp.8**	Wilk's λ	0.88	0.91	0.86	0.95	0.84	0.93
	$F_{1, 4}$	12.9	2.3	0	12.1	0.3	5.6
	P	0.001	0.14	0.932	0.001	0.56	0.023
***Phintella* sp.14**	Wilk's λ	0.55	0.94	0.79	0.72	0.82	0.95
	$F_{1, 4}$	12.6	2.3	0	12.4	24.6	4.1
	P	0.001	0.139	0.889	0.001	<0.001	0.052

2.3.2.3 Sexual dichromatism

Intersexual spectral reflectance variations in the four main body regions of the nine *Phintella* species are shown in **Figure 2.2**. Intersexual difference details in the abdomen (dorsal and lateral) are presented in **Table 2.8**. Overall, the dorsal abdomen showed significant intersexual differences than the lateral abdomen. More species showed sexual dichromatism in the UV range (seven species) than in the VIS range (5 species) in the dorsal abdomen. In addition, significant intersexual variations happened more often in the Qt and chroma than in the hue for both the UV and VIS ranges **(Fig. 2.3).**

Intersexual differences on the carapace (dorsal and lateral) are presented in **Table 2.9.** On the carapace, the lateral carapace showed as more intersexual differences as the dorsal carapace. The intersexual variations in the UV hue usually presents similar between the dorsal and lateral carapace **(Fig. 2.4).**

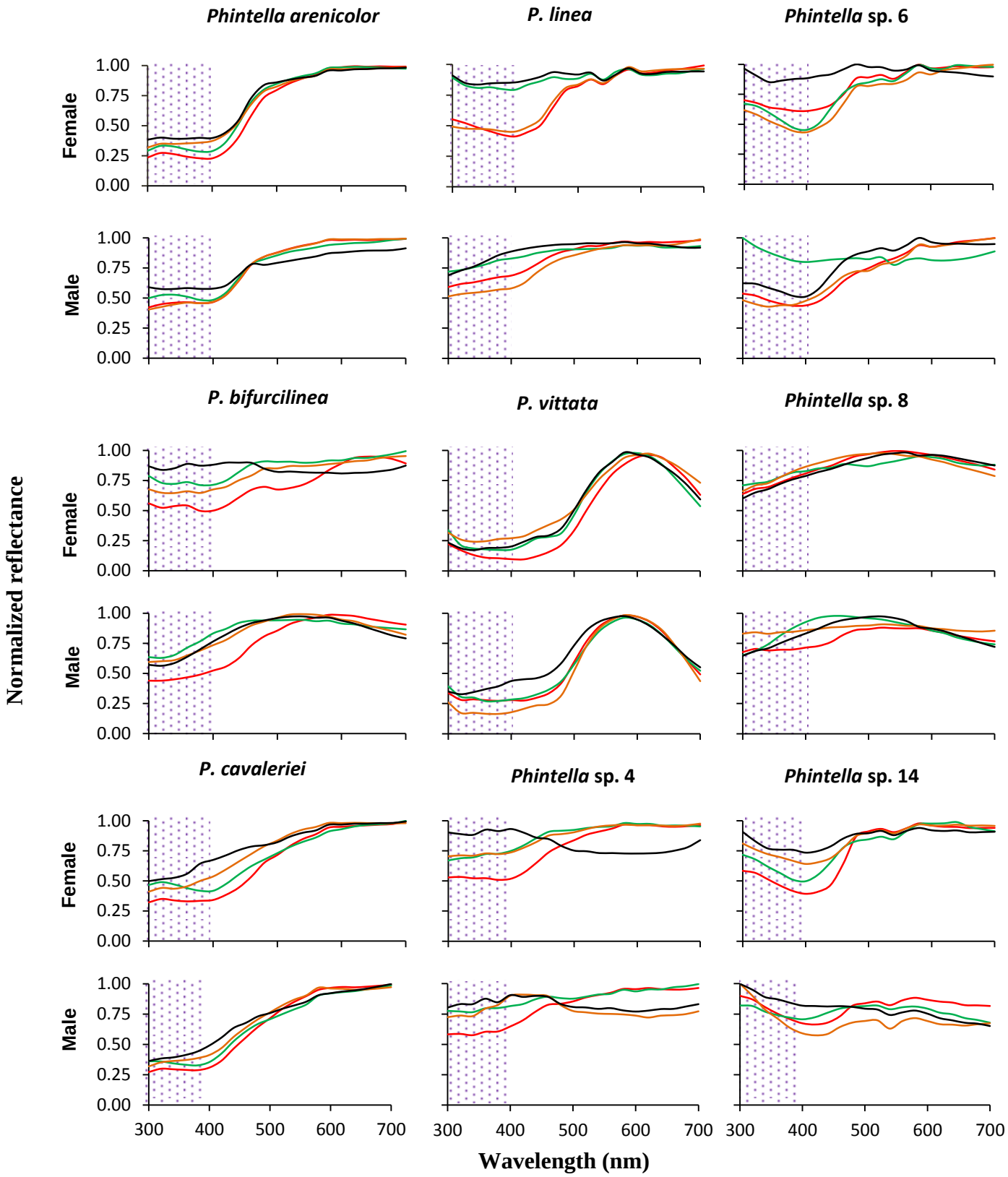


Figure 2.2 Mean reflectance spectra (normalized) of four main body regions (red curve: dorsal abdomen, green curve: dorsal carapace, yellow curve: dorsal abdomen, black curve: dorsal carapace) measured from nine *Phintella* species for both females and males (N = 5 for each gender of each species). Shaded area is UV reflectance range (wavelength 300-400 nm) and unshaded area is VIS reflectance range (wavelength 400-700 nm).

Table 2.8. Sexual dichromatism in the body region of abdomen (dorsal and lateral view) for each of the nine *Phintella* species. Blanked value in some hue means there is no peak exist in that wavelength range.

Species		Dorsal abdomen						Lateral abdomen					
		UV			VIS			UV			VIS		
		Qt	Hue	Chroma	Qt	Hue	Chroma	Qt	Hue	Chroma	Qt	Hue	Chroma
	Wilk's λ	0.31	0.53	0.51	0.64	0.8	0.62	0.31	0.2	0.53	0.53	0.42	0.49
P. arenicolor	$F_{1, 4}$	7.93	3.14	8.61	665.2	166.8	411.14	5.91	0.05	0.47	4.01	0.02	4.04
	P	0.023	0.114	0.019	<0.001	<0.001	<0.001	0.041	0.824	0.513	0.08	0.886	0.079
	Wilk's λ	0.86	0.53	0.67	0.72	0.1	0.67	0.54	0.71	0.73	0.22	0.38	0.31
P. bifurcilinea	$F_{1, 4}$	0.82	4.19	0.02	5.19	3.74	0.75	0.03	9.23	1.35	2.48	6.52	1.53
	P	0.393	0.075	0.906	0.052	0.089	0.411	0.86	0.016	0.279	0.154	0.034	0.252
	Wilk's λ	0.56	0.74	0.33	0.68	0.35	0.37	0.48	0.41	0.56	0.41	0.57	0.72
P. cavaleriei	$F_{1, 4}$	0.63	0.88	8.19	0.02	0.16	0.03	2.51	5.23	0	3.48	0.62	3.38
	P	0.45	0.376	0.021	0.886	0.7	0.877	0.152	0.051	0.951	0.099	0.454	0.103
	Wilk's λ	0.64	0.72	0.57	0.74	0.39	0.46	0.57	0.54	0.66	0.21	0.4	0.78
P. linea	$F_{1, 4}$	9.69	11.35	5.65	12.68	0.28	21.37	1.5	2.13	0.25	1.84	1.14	2.71
	P	0.014	0.01	0.045	0.007	0.612	0.002	0.255	0.183	0.63	0.212	0.317	0.138
	Wilk's λ	0.36	0.69	0.73	0.53	0.38	0.72	0.69	0.35	0.16	0.51	0.83	0.65
P. vittata	$F_{1, 4}$	1.21	0.53	5.48	2	0.19	2.33	2.71	2.16	0.47	1.46	8.38	1.82
	P	0.303	0.488	0.047	0.195	0.677	0.166	0.139	0.18	0.512	0.262	0.02	0.214
	Wilk's λ	0.56	0.43	0.16	0.27	0.3	0.52	0.58	0.53	0.71	0.52	0.69	0.6
Phintella sp. 4	$F_{1, 4}$	0.53	3.97	1.81	0.62	2.42	0.83	0.72	1.64	3.85	9.62	3.85	1.49
	P	0.489	0.082	0.215	0.455	0.158	0.389	0.422	0.236	0.085	0.015	0.085	0.257
	Wilk's λ	0.07	0.26	0.78	0.02	0.06	0.54	0	.	0.15	0.01	0.01	0.02
Phintella sp. 6	$F_{1, 4}$	60.32	13.62	17.57	89.53	105900	56.03	1.69	.	9.4	0.45	2.02	0.22
	P	<0.001	0.006	0.003	<0.001	<0.001	<0.001	0.23	.	0.015	0.523	0.193	0.652
	Wilk's λ	0.52	0.59	0.36	0.47	0.32	0.59	0.66	0.73	0.32	0.73	0.56	0.41
Phintella sp. 8	$F_{1, 4}$	1571	13.88	0.34	9.51	0.07	5.46	1.49	5.45	69.65	6.79	1.46	3.96
	P	<0.001	0.007	0.578	0.018	0.804	0.052	0.257	0.048	<0.001	0.031	0.261	0.082
	Wilk's λ	0.12	.	0.3	0.53	0.54	0.77	0.05	.	0.2	0.04	0.6	0.7
Phintella sp. 14	$F_{1, 4}$	9.34	.	1.04	1.58	0.54	7.4	1	.	21.14	5.75	2	0.06
	P	0.016	.	0.338	0.245	0.485	0.026	0.347	.	0.002	0.043	0.196	0.817

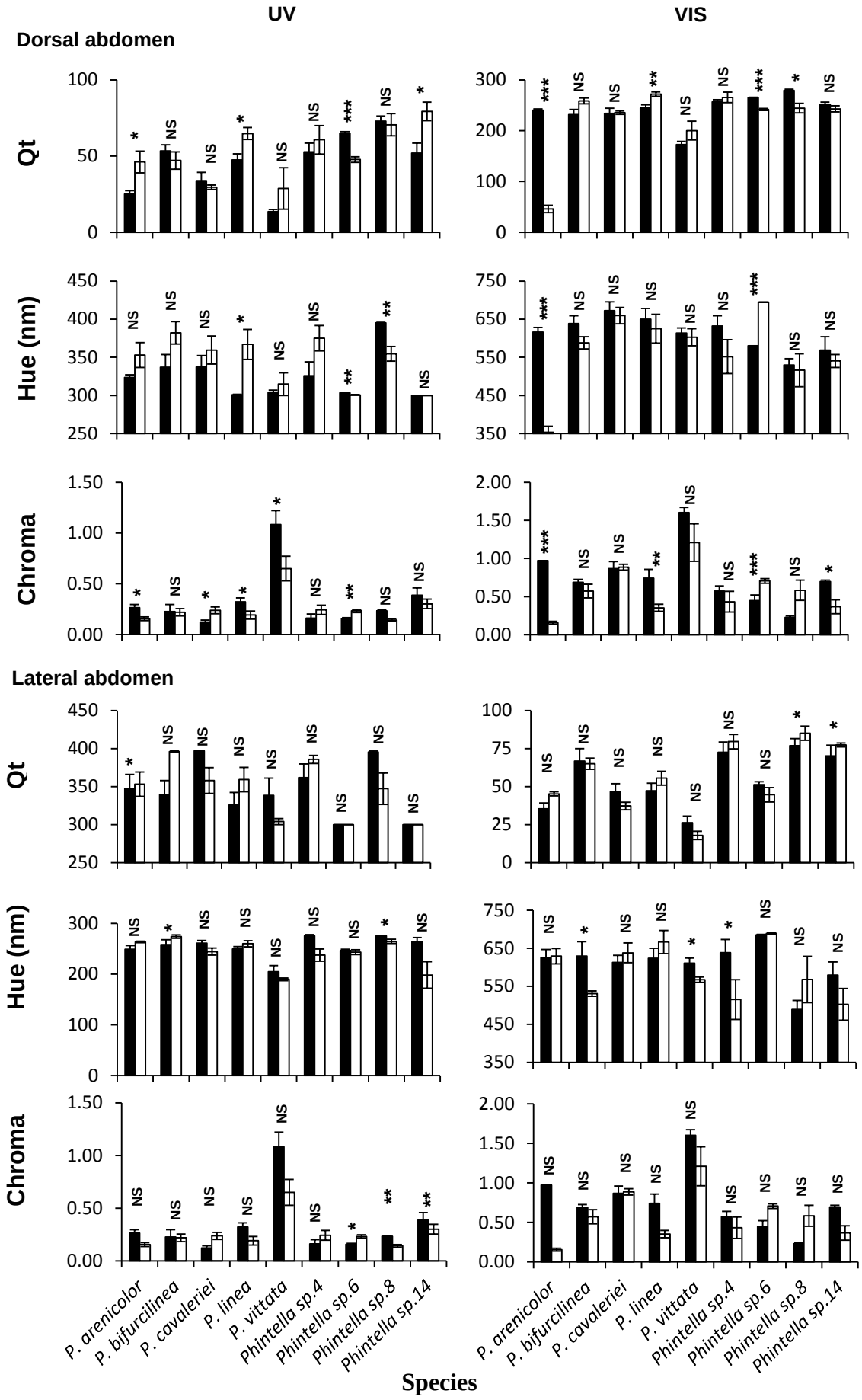


Figure 2.3. Sexual dichromatism details in the body region of abdomen (both dorsal and lateral view) in the nine *Phintella* species. Black bar female, white bar: male. NS: no significant difference, *: $P < 0.05$, **:$P < 0.01$, ***: $P < 0.001$.

Table 2.9. Sexual dichromatism in the body region of carapace (dorsal and lateral view) for each of the nine *Phintella* species. —: no peak exist in that wavelength range.

Species		Dorsal carapace						Lateral carapace					
		UV			VIS			UV			VIS		
		Qt	Hue	Chroma	Qt	Hue	Chroma	Qt	Hue	Chroma	Qt	Hue	Chroma
	Wilk's λ	0.52	0.24	0.63	0.47	0.52	0.51	0.6	0.54	0.55	0.62	0.62	0.57
P. arenicolor	$F_{1, 4}$	59.74	1.45	0.32	0.59	0.4	54.98	2.7	0.19	0.02	103.6	17.24	14.1
	P	<0.001	0.263	0.586	0.463	0.543	<0.001	0.139	0.674	0.907	<0.001	0.003	0.006
	Wilk's λ	0.79	0.19	0.25	0.41	0.61	0.57	0.29	0.44	0.67	0.45	0.64	0.76
P. bifurcilinea	$F_{1, 4}$	0.28	15.45	2.52	0.44	6.18	2.69	7.61	5.9	11.21	8.45	0.09	1.37
	P	0.613	0.004	0.151	0.527	0.038	0.14	0.025	0.041	0.01	0.02	0.774	0.276
	Wilk's λ	0.53	0.75	0.73	0.73	0.88	0.67	0.64	0.52	0.53	0.44	0.48	0.27
P. cavaleriei	$F_{1, 4}$	4.83	0.61	0.42	0.8	2.26	1.98	3.11	0.29	0	4.18	4.89	3.99
	P	0.059	0.459	0.536	0.396	0.171	0.197	0.116	0.603	0.973	0.075	0.058	0.081
	Wilk's λ	0.76	0.03	0.73	0.55	0.44	0.33	0.62	0.57	0.48	0.23	0.42	0.8
P. linea	$F_{1, 4}$	0.32	1337	0.28	0.22	2.47	0.13	2.01	21.63	128.23	0.63	0.24	0.4
	P	0.585	<0.001	0.611	0.651	0.155	0.729	0.194	0.002	<0.001	0.45	0.639	0.543
	Wilk's λ	0.21	0.75	0.4	0.26	0.11	0.19	0.56	0.6	0.48	0.72	0.33	0.52
P. vittata	$F_{1, 4}$	1.97	2.67	1.94	0.97	1.5	1.37	6.21	0.4	0.57	3.04	815.46	4.82
	P	0.198	0.141	0.202	0.354	0.256	0.276	0.037	0.546	0.474	0.119	<0.001	0.059
	Wilk's λ	0.43	0.35	0.84	0.82	0.48	0.53	0.57	0.44	0.8	0.47	0.41	0.49
Phintella sp. 4	$F_{1, 4}$	1.5	0.01	0.12	0.72	1.04	0.78	5.36	4.89	0.11	0.47	0.64	0.11
	P	0.256	0.92	0.733	0.422	0.337	0.404	0.049	0.058	0.755	0.514	0.449	0.752
	Wilk's λ	0	0.01	0.05	0	0.16	0.1	0.03	0.01	0.13	0.01	0.01	0.12
Phintella sp. 6	$F_{1, 4}$	139.3	44.55	17.95	7.78	1174	112.11	696.3	20.6	51.98	332.9	4636000	643.2
	P	<0.001	<0.001	0.003	0.024	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001
	Wilk's λ	0.67	0.56	0.45	0.59	0.61	0.56	0.76	0.6	0.79	0.6	0.3	0.67
Phintella sp. 8	$F_{1, 4}$	0.02	17.69	5.53	0.01	4.13	0	0.13	1	0	5.31	0.49	1.13
	P	0.883	0.003	0.047	0.944	0.077	0.948	0.73	0.347	0.99	0.05	0.503	0.319
	Wilk's λ	0.27	0.75	0.6	0.32	0.57	0.58	0.09	—	0.53	0.03	0.65	0.18
Phintella sp. 14	$F_{1, 4}$	1.56	2.62	23.97	3.03	26.85	2.99	3.99	—	0.02	3.16	21.22	0.01
	P	0.247	0.144	0.001	0.12	0.001	0.122	0.081	—	0.896	0.114	0.002	0.944

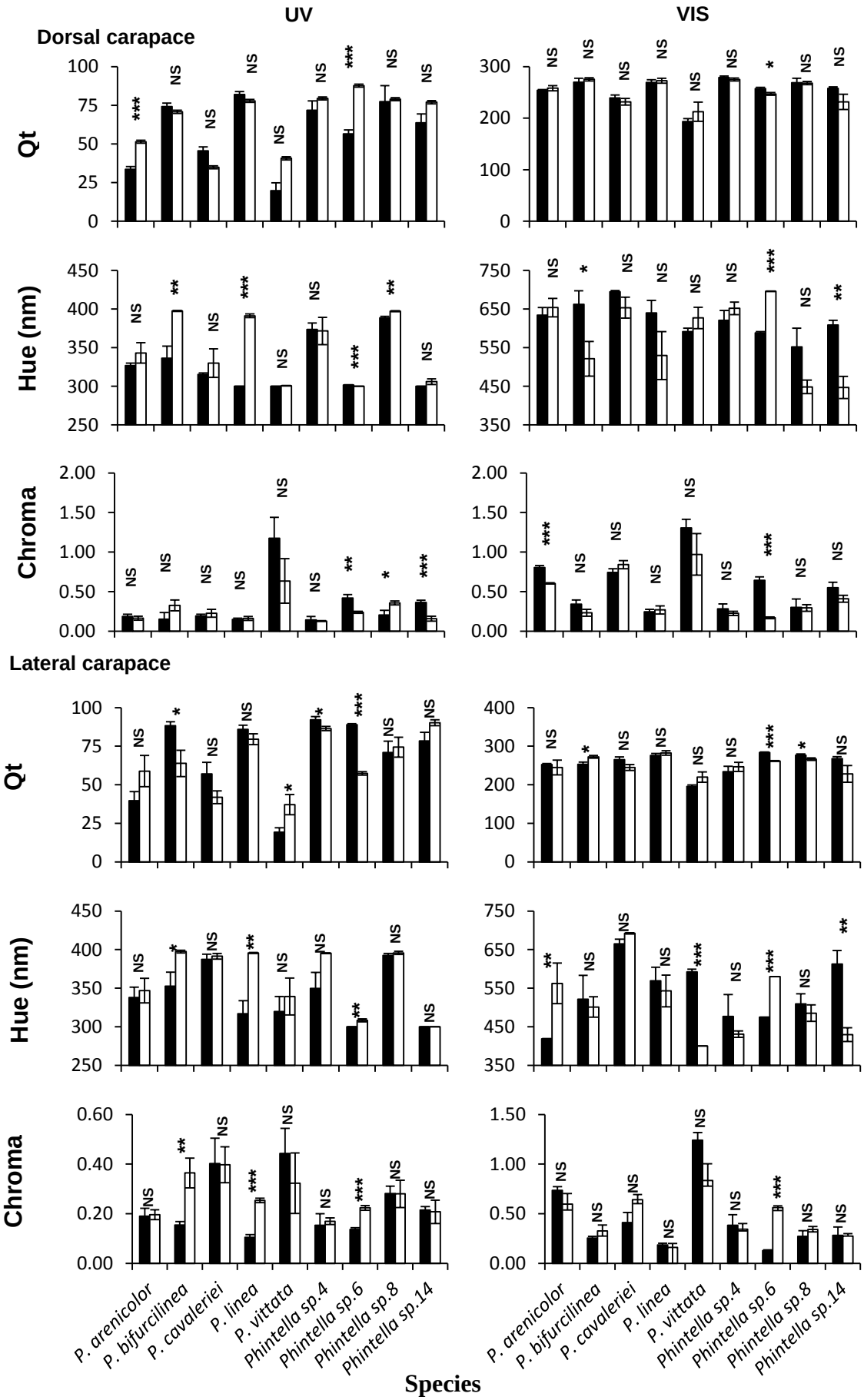


Figure 2.4. Sexual dichromatism in the body region of carapace (both dorsal and lateral view) in the nine *Phintella* species. Black bar: female, white bar: male. NS: no significant difference, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

2.4 Discussion

This is the first comparative study to investigate colouration variation in multiple closely related species in jumping spiders. Our results have shown considerable inter- and intraspecific variations in both UV and VIS wavelength ranges. The results are necessary to trace back the evolutionary history of colouration in these jumping spiders, for example, how the colour changes across different species, different body regions and sexual dichromatism or which of the three colour components (total brightness, hue and chroma) are more likely to change and which is relatively conservational when mapping the colouration onto the phylogenetic tree. Combined with behavioural studies, the correlation between colour evolution and speciation event also can be investigated.

This study suggests that sexual dichromatism is a common phenomenon in the genus of *Phintella*. I found that all the nine species examined showed colour differences between males and females. The sexual dichromatism presented in both the reflectance bands (UV and VIS); *Phintella* species tested showed sexual dichromatism in the UV range as often as in the VIS range. These results are in accordance with previous studies in the salticid species *Cosmophasis umbrutica* (Lim and Li, 2006b) and *P. vittata* (Li et al., 2008c; Lim et al., 2008a), both of which show sexual dichromatism in both UV and VIS ranges as well as in multiple body regions. However, to date a limited number of salticid species have been studied for sexual dimorphism in UV colour and other colour ranges. Furthermore, behavioural studies have demonstrated that UV sexual dichromatism usually plays a key role in female mate choice (Li et al., 2008c; Lim et al., 2008a) and intra-sex male competition (Lim and Li, 2006a). Therefore, in order to investigate the widespread and behavioural

functions of UV and VIS colours, more studies are needed on UV and VIS sexual dimorphism in salticids.

Phintella females commonly showed stronger spectral reflectance in the brightness or chroma than males. This result contradicts with previous studies claiming that males often show brighter ornamental colouration than females (Richman and Jackson, 1992). I believe that either males brighter than females or females brighter than males are common in animals, but most of the previous studies deliberately focussed on sexually selected ornamental colours in males for investigation, which may explain why only a single exception was found (Forster, 1982). I assume that different colourations may play role in different aspects of either natural or sexual selection. For example, UV-green iridescence can be an indicator of a male's quality (Lim and Li, 2013a), whereas blue colours can be indicator of prey items for salticids (Taylor et al., 2014). Therefore, most probably, males are not necessarily brighter than females in non-sexual selected colourations.

As shown in this study, there is a considerable intersexual difference in brightness, chroma and hue for each specific body region. For a specific spectral reflectance, usually just one or two of the three colour components (brightness, chroma and hue) differ significantly between the sexes, or the significance level often quite different among the three colour components. These results imply that the evolutionary process of brightness, chroma and hue could be independent or different. Furthermore, it is still worth exploring whether the three colour components play roles in different aspects of the species biology. The idea is supported by a recent study (Lim et al., 2008a), as they pointed out that *C. umbratica* females specifically use hue but not brightness of a male's UV colouration to select their mates.

There are two main limitations of this study. The first one is that only nine out of 54 *Phintella* species (World Spider Catalog, 2015) were used in this investigation. This will be a limitation when tracking the evolution of colour on a phylogenetic tree. Therefore, more species should be used from the genus of *Phintella* for quantifying sexual dichromatism and its evolution in the future. The second limitation is that colour variation between geographical populations of some species were not investigated, although population variation in colours commonly exists from our field observations, especially in males (pers. obs.). This also has been shown by a previous study in a salticid species *P. vittata* in the UV spectral reflectance band (Li et al., 2008a). As between-population differences usually occur only in one or two colour morphs, it is relatively easy to analyse the connections between colour change and the explanatory factors compared with directly analysing between species colouration changes. Hence our suggestion for future studies is to research sexually dichromatic colouration among different geographic populations within a particular species, and then to analyse the according factors (can be sexual factors or ecological factors) of the among-population colour change.

CHAPTER 3

Effects of UV-reflecting markings on mate-choice in eight species of the jumping spider genus *Phintella* (Araneae: Salticidae)

3.1 Introduction

Colouration is known usually as a clue for females to select a potential mate in numerous animals such as birds, fish, reptiles and insects. Evidence shows that colouration-connected direct or indirect benefits in enhancing reproductive success may be the engine of colour-based mate choice in many animals (Blount et al., 2003; García-González and Simmons, 2007; Griffith and Pryke, 2006; Karino et al., 2005; Reynolds and Gross, 1990). Overwhelmingly majority of previous studies have focused on colour-based female mate-choice, but most of these studies are investigating human-visible colours (VIS, wavelength: 400–700 nm) (Clark and Uetz, 1993; Hill, 1990; Houde, 1987; Suarez-Gonzalez and Cassini, 2013). Ultraviolet (UV; wavelengths between 280–400 nm) colour, to which humans are blind (Bennett et al., 1994; Burkhardt and Maier, 1989; Cuthill and Bennett, 1993), has been the focus of many recent studies of visual communication in a wide range of animals. These investigations have shown that UV reflection (Burkhardt and Maier, 1989; Finger, 1995; Lim and Li, 2006b; Mullen and Pohland, 2008) and sensitivity to UV (Lim and Li, 2006a; Lim et al., 2007b; Olofsson et al., 2010) are much more widespread in animals than previously assumed. A growing body of research has demonstrated that UV visual signals are involved in sex recognition (Guillermo-Ferreira et al., 2014; Lim et al., 2007b; Ries et al., 2008), intrasexual competition in both vertebrates (Bajer et al., 2011; Rick and Bakker, 2008; Siefferman and Hill, 2005; Stapley and Whiting, 2006) and invertebrates (Detto and Backwell, 2009; Lim and Li, 2006a; Lim and Li, 2013b; Xu and Fincke, 2015), and female mate-choice in both vertebrates (Andersson, 1999; Bajer et al., 2011; Hunt et al., 1998b; Kurvers et al., 2010) and invertebrates (Detto and Backwell, 2009; Kemp, 2008; Knüttel and Fiedler, 2001; Li et al., 2008b). Yet, few comparative studies have been conducted to investigate adaptive functioning

of UV reflection across multiple closely related species. Moreover, there has been little to no evidence of UV-based male mate-choice in animals.

Jumping spiders (Salticidae) is the largest family with 5794 species (World Spider Catalog, 2015) and they have complex courtship display behaviour and obvious sexual dichromatism. Salticids have excellent vision (Blest et al., 1990; Land, 1969a, 1985; Land and Nilsson, 2012). It is widely accepted that UV is one of the primary colours of the salticid principal eye, with UV-sensitive cells in Layers IIII and IV having peak sensitivity at 330–380 nm (De Voe, 1975a; Peaslee and Wilson, 1989; Yamashita and Tateda, 1976). However, it is only recently that details concerning how salticids use UV have been clarified and the results have shown that some salticid species have strikingly iridescent markings (Li et al., 2002; Lim and Li, 2006a; Li et al., 2008a). Iridescence is of particularly interest because we have shown that it often adds UV colouration to the salticids, exhibiting UV sexual dichromatism, with only males reflecting UVA, UVB light or both (Lim and Li, 2004, 2006a; Lim et al., 2008b). The work from behavioural experiments has confirmed that they are capable of seeing both UVA and UVB (Li et al., 2008b; Lim and Li, 2006a, b; Lim et al., 2007b; Lim et al., 2008b). A few studies have provided further evidence that UVA (Li et al., 2008a; Lim et al., 2007b) and UVB (Li et al., 2008b) signals serve as a criterion used by females when making mate-choice decisions. Surprisingly, however, UV reflection and its adaptive significance have been investigated in only two (*Cosmophasis umbratica* and *Phinetlla vittata*) (Lim and Li, 2006b, Li et al., 2007; Li et al., 2008a, b; Lim et al., 2008) of 5794 described species (World Spider Catalog, 2015). Moreover, no study has investigated into adaptive functioning of UV reflection across multiple closely related species. Nevertheless this sort of study is necessary for objectively assessing the widespread and function of UV in salticids. Such a

comparative study may also shed light on how male UV colour and UV-based female mate-choice occurred in the first place and how UV-based female mate-choice have evolved and maintained. Furthermore, little is known about whether UV is important in male mate-choice, though females can usually have similar or even brighter in the UV reflectance than males in some species **(Chapter 2)**.

Salticids of the genus *Phintella* that contains more than 50 species (World Spider Catalog, 2015) are widely distributed in tropical, sub-tropical and temperate area. Spiders of *Phintella* are usually small to medium (3-6 mm in body length). Some tropical species are usually covered with metallic iridescent scales, which exhibit ornamented structure colours (Matsumoto, 1989), including UV reflection (Li et al., 2008a). However, surprisingly the functioning of UV reflection has only been tested in *P. vittita*, a highly ornamented species distributed in tropical East and South-east Asia (Li et al., 2008b), in which male UV reflection has been demonstrated to be used by females in making mate choice decision. In Chapter 2, we quantified inter- and intraspecific variations in colouration, including UV, in nine species *Phintella* from Asia (**Fig. 3.1**). The results from Chapter 2 showed that: i) UV reflectance commonly exists in these nine species and in both males and females; ii) There are considerable variations in spectral reflectance between species, and between sexes; iii) All the nine species show sexual dichromatism in spectral reflectance in both UV and VIS wavelength ranges. The goal of the present study was to tease apart the hypotheses for the possible functions of colour in intraspecific communication across the eight closely related species of *Phintella*. Specifically, we examined whether UV reflection is more likely used in female mate-choice or male mate-choice (mutual mate choice) and whether UV-based male choice is species specific.

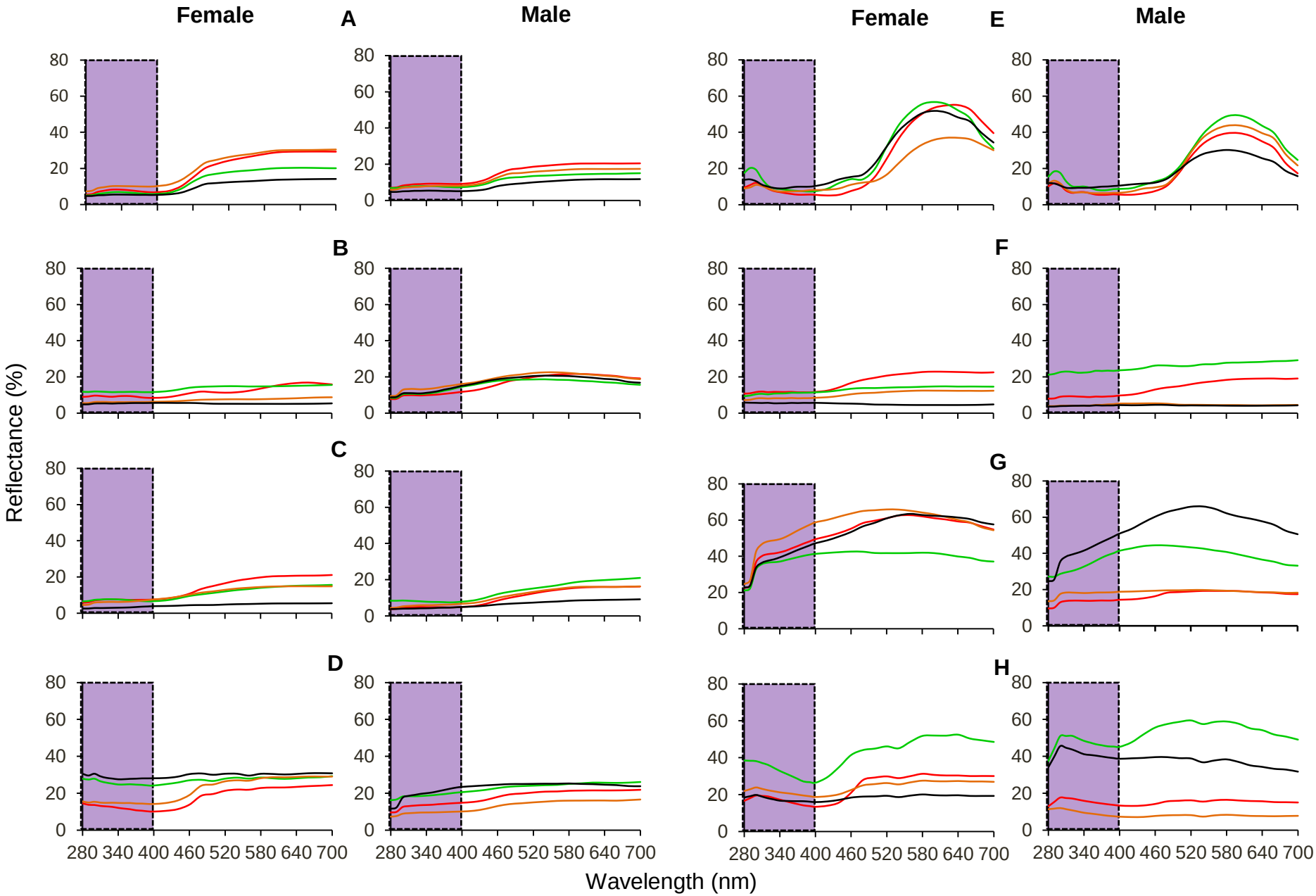


Figure 3.1. Spectral reflectance of the eight *Phintella* species used in study. **(A)** *P. arenicolor*; (**B**) *P. bifurcilinea*, **(C)** *P. cavaleriei*; (**D**) *P. linea*; (**E**) *P. vittata***; (F)** *Phintella* sp. 4**; (G)** *Phintella* sp. 8**;** and **(H)** *Phintella* sp. 14. Shaded area: UV wavelength range (280-400 nm); unshaded area: VIS wavelength range (400-700 nm). Red line: dorsal abdomen (DA); green line: dorsal carapace (DC); orange line: lateral abdomen (LA); black line: lateral carapace (LC).

3.2 Materials and methods

3.2.1 Study subjects and maintenance

Eight species of *Phintella* that were collected from East and South-east Asia were used in this study **(Table 3.1)**. Spiders were collected either as adults or juveniles by beating bushes and shrubs, and then transported them to the laboratory at the National University of Singapore (Singapore). They were kept individually in plastic cylindrical cages (diameter x height: 60 mm x 80 mm) and maintained in a laboratory with controlled environmental conditions (relative humidity: 80-90%; temperature: 25 ± 1 ℃; light regim: 12:12 h light/dark cycle; lights on 08:00 h) using a standard protocol as used in early salticid studies (Lim and Li, 2004, 2006a, b; Li et al., 2008a, b). Opaque white sheets of paper were inserted between cages to prevent visual interactions with neighbouring conspecific individuals. Fruit flies (*Drosophila melanogaster*) were provided to them twice a week as food. Spiders collected as juveniles were monitored every two days until they reached sexual maturation (i.e. the last moult) so that their post- maturation age was known.

Table 3.1. The information on the locality of eight species of *Phintella* used in the study.

Species	Collected location	Latitude (N) / Longitude(E) / a.s.l. (m)
Phintella arenicolor	Changsha, Hunan, China	28°15'/ 112°14' / 118
P. bifurcilinea	Xishuangbanna Tropical Botanic Garden (XTBG), Yunnan, China	21°56'/ 101°16'/ 571
P. cavaleriei	Erlangping, Xixia county, Nanyang, Henan, China	33°32' / 111°41' / 501
P. linea	Jiugongshan, Tongshan County, Xianning, Hubei, China	29°25' / 114°40' / 1129
P. vittata	Xishuangbanna Tropical Botanic Garden (XTBG), Yunnan, China	21°54' / 101°15' / 559
Phintella sp. 4	Xishuangbanna Tropical Botanic Garden (XTBG), Yunnan ,China	21°56' / 101°15' / 565
Phintella sp. 8	Xishuangbanna Tropical Botanic Garden (XTBG), Yunnan, China	21°53' / 101°14' / 577
Phintella sp. 14	Northern University of Malaysia (UUM) Sintok, Kedah, Malaysia	6°28' / 100°30' / 277

3.2.2 Experimental procedure

We used a mutual mate-choice design to test both female and male mate-choice simultaneously using a single dichotomous choice design with an apparatus consisting of three separate glass chambers (**Fig. 3.2**). One large female chamber holding a female was placed facing two smaller male chambers, each holding a courting male. All the seven sides of the apparatus was made of microscope slides covered with black paper from the outside surface so that light could not transmit through them from the chamber sides into the chambers. The full-spectrum transmitting quartz glass slide (model: JGS1) was used to separate female chamber from two male chambers. As both UV and VIS light could pass through the quartz glass slide, it made a mutual assessment possible between the female and the male based on their spectral reflectance of both UV and VIS ranges (UV+ male and UV+ female). To create UV-blocked male or UV-blocked female, we placed a sheet-like UV-blocking filter between the interface of the female chamber and one of the male chambers at random. The UV filter blocked the transmission of all light below 400 nm across the chamber, resulting in an UV filtered environment in one of the male chamber (UV– male: filter present) from the female's view or in the female chamber (UV– female: filter present) from the male's view so that the mutual assessment between UV– male and UV– female could be achieved only based on their VIS reflectance. In all trials, the filter was randomly placed across either the left or right chamber to rule out any side preference effects of the female. The apparatus was covered by a quartz glass at top during the trials to prevent spiders from escaping.

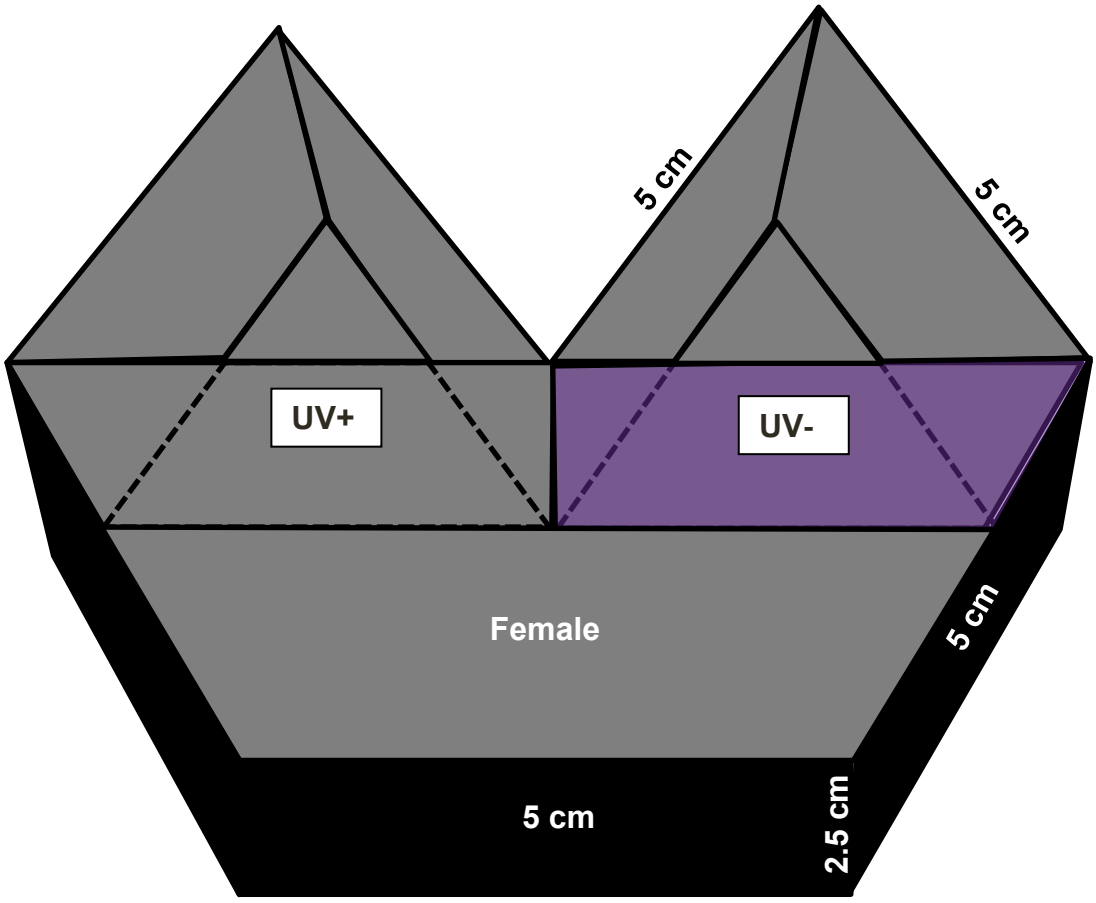


Figure 3.2. Mate-choice apparatus used in mate choice trials and filter transmission spectra. **(A)** Frontal 3-D view of three glass chambers. One holding a female (‘female chamber’), and two holding two males each (UV+ and UV– ‘male chambers’). **(B)** Transmission spectra demonstrating light wavelengths transmitted through glass with filter and light used in experiments: no filter (UV+); UV-blocking filter (UV–). Spectra were measured with Ocean Optics USB4000 UV/VIS miniature fibre-optic spectrometer (Ocean Optic Inc., Dunedin, USA) and DH-2000 deuterium tungsten halogen light source (Ocean Optics Inc., Dunedin, USA)

Males paired against one another in each trial were matched according to body mass (to nearest 0.1 mg), body length (BL) and carapace width (CW) (to nearest 0.01 mm). These parameters were obtained one day before the mate choice trials and no significant difference was found in colour or size between males used in the trials. Spiders collected as adults were tested between two to seven days after their collection, and spiders collected juveniles were tested within 2-3 weeks after they reached maturity in the laboratory. Each mate-choice trial comprised of two phases: (1) female acclimatisation phase, and (2) mate assessment phase. We did not conduct the control phase as previous studies have shown that light environment and side have no significant effect on salticid behaviour (Li et al., 2008a; Li et al., 2008b). The five-minute female acclimatisation phase began when the female was introduced into its chamber. During this phase, the male chambers were empty and were blocked from view with black cardboard, which was placed between the male and female chambers. The mate assessment phase started upon the retraction of the opaque cardboard between the male and female chambers and lasted for 10 mins. Females used in all trials were chosen from the same post-maturation age group. Enclosure walls were cleaned with 75% ethanol and distilled water after each trial to remove any chemical pheromones present. All trials were performed between 0800 to 1800 h to coincide with the active periods of the spiders. No female was reused and no two males were paired together more than once. All the trails were carried out under 10 Voltarc Ultra tubes (110 W each, powered by a 120 V50/60 Hz electronic ballast; SUPER-TEK, Naturallighting.com, Houston, Texas) and 2 additional UV light emitting dark tubes (Hitachi BL/B, 20W each, powered by a 230 V 50/60 Hzelectronic ballast), all of which were suspended about 150 cm above the apparatus. All interactions were video recorded using a high definition digital video camera (JVC GZ-MG50AG) that was

placed through a slit in a surrounding black curtain to minimise disturbance to the spiders (Li et al., 2008b). Video playbacks of all interactions, set at highest image resolutions per frame, were subsequently analysed blindly with regard to light treatment at a resolution of 25 frames per second (Videolan media player for Windows).

To determine female mate preference we used the time spent by the female watching a particular male (i.e. female attention), which was defined as the time when she had the gaze of her anterior median eyes orientated directly towards a male (Hebets and Maddison, 2005). This was determined based on the directional view of the carapace (Lim et al., 2008b). To determine male mating preference, we recorded the time spent by each male displaying courtship postures during the mate assessment phase.

3.2.3 Data analysis

For female mate-choice trials where possible we compared the response (i.e., female attention) of females to males lacking UV light using paired *t*-tests or Wilcoxon signed-rank tests when the data were not normally distributed or sample size was small. For male mate choice, we also used paired *t*-tests or Wilcoxon signed-rank tests to compare the time males spent displaying the courtship behaviour to UV+ females and UV– females. All statistical analyses were performed using SPSS 22 (IBM SPSS).

3.3 Results

3.3.1 Female mate-choice

In general, in all the species examined except *Phintella* sp. 14, females spent longer time watching UV+ males than watching UV– males, but only *P. vittata* and *Phintella*

sp. 8 females spent significantly longer time watching UV+ males than watching UV– males (**Fig. 3.3**). *Phintella cavalariei* females tended to spend longer time looking at UV+ males than watching UV– males, but the difference in female attention (i.e., time the female spent watching a courting male) was marginally non-significant. In contrast, *Phintella* sp. 14 female spent longer time watching UV– males than UV+ males though there was no significant difference. Females of four species (*P. arenicolor, P. bifurcilinea, P. linea* and *Phintella* sp. 4) spent as much time watching UV+ males as they spent watching UV– males.

3.2.2 Male mate-choice

Phintella cavaleriei, P. vittata and *Phintella* sp. 8 males displayed courtship behaviour to females significantly longer when the UV filter was absent than when the UV filter was present (**Fig. 3.4**). Conversely, *Phintella* sp. 14 males tended to display courtship behaviour to females longer when the UV filter was present than when the UV filter was absent in spite of no significant difference. Males of other four species (*P. arenicolor, P. bifurcilinea, P. linea* and *Phintella* sp. 4) displayed the similar time when the UV filter was present as when the UV filter was absent.

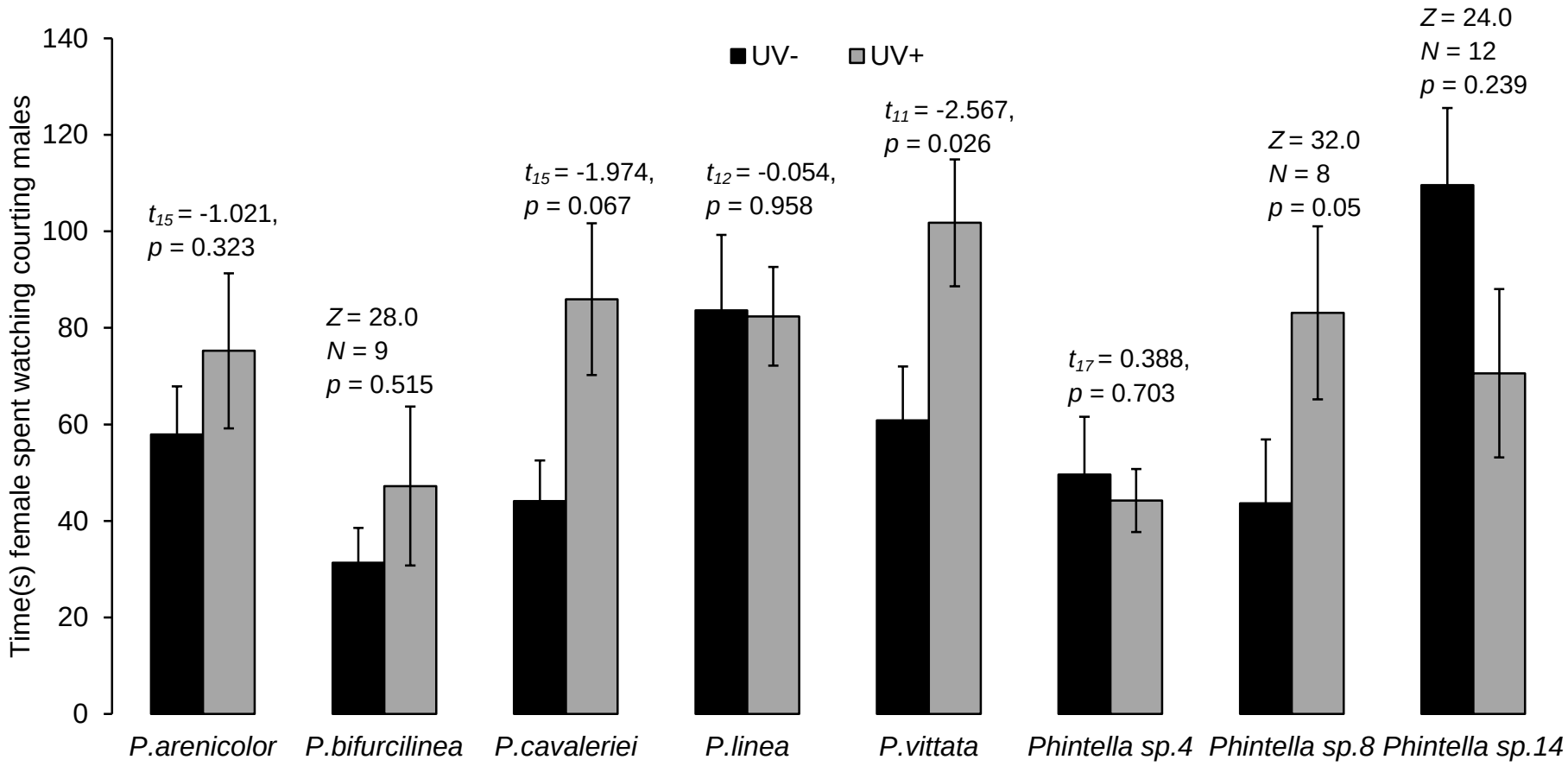


Figure 3.3. Mean (± SE) time (s) female spent watching courting UV+ or UV–males during mate-assessment phase in the eight *Phintella* species.

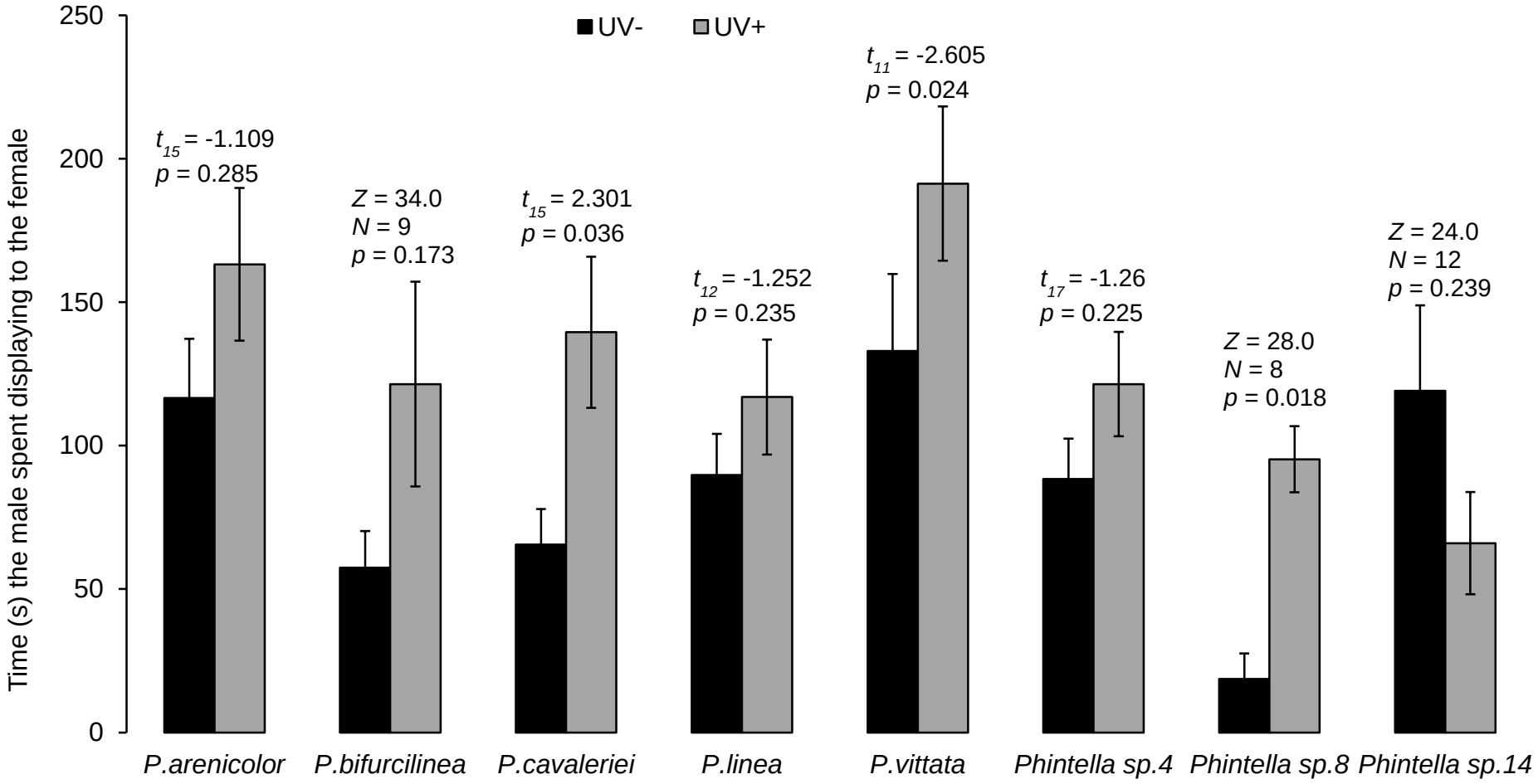


Figure 3.4. Mean (± SE) time (s) males spent displaying courtship behaviour to UV+ or UV– females during mate-assessment phase in eight species of *Phintella*.

3.4 Discussion

In Chapter 2, inter- and intraspecific variations in colouration, including UV, were investigated in nine species of *Phintella* from Asia. The results revealed the existence of the UV reflection, considerable variation in spectral reflectance between species, and the presence of sexual dichromatism in both UV and VIS wavelength ranges in all the nine species. In this study, we examined whether UV reflection is more likely used in female mate choice or male mate-choice, and whether UV-based male choice is species specific. Our results showed that though all eight *Phintella* species that were examined had body regions that reflect UV light (Chapter 2), not all species used UV reflection for choosing mates; tropical species were more likely to use UV reflection in their mate choice compared to temperate species. We also found that *Phintella* species (*P. cavaleriei*, *P. vittata* and *Phintella* sp. 8) were more likely to use UV reflection in male mate-choice than in female mate-choice (*P. vittata* and *Phintella* sp. 8). This also provided the first evidence of UV reflection used in male mate-choice in jumping spiders. In *P. vittata* and *Phintella* sp. 8, both males and females used the UV reflection in choosing their mates, thus the evidence of mutual mate choice. In contrast, *Phintella* sp. 14 seemed to use human-visible rather than UV colour in their mutual mate-choice. This is the first study to investigate functioning of UV reflection in mate choice across multiple closely related species in jumping spiders.

Our results indicated that tropical *Phintella* species are more likely than temperate species to use UV reflection in their mate-choice. *Phintella vittata*, *Phintella* sp. 8 and *Phintella* sp. 14 are distributed in tropical South-east and East Asia (World Spider Catalog, 2015), where UV radiation is relatively long and strong than many temperate regions (Lythgoe, 1979). In addition, UV wavelengths are more

strongly scattered in air and water than longer wavelengths (Lythgoe, 1979), thus favouring communication over short distances for small animals such as salticids with the signal being difficult to detect by more distant perceivers such as predators (Bennett and Cuthill, 1994). Therefore, salticid species in tropics have body surface that reflect UV so that they can avoid damages caused by UV radiation of sunlight in the first place, and later may in their evolutionary history have used UV reflection for intraspecific communication such as sex recognition and mate choice. As the main habitats of *Phintella* species are mainly shrubs and bushes that are exposed under glare, it should be vital for females, males or both to select the best mates (individuals have maximum UV radiation) for their offspring. Hence we suppose that this adaptive selection may have resulted in UV-based mate-choice in these tropical salticids. However, species from temperate regions (i.e., *P. arenicolor, P. linea*) usually appear dull in colour with lower UV reflectance, thus UV reflection may not be crucial in mate choice. *Phintella bifurcillinea* and *Phintella* sp. 4 are distributed in tropics, but our results showed that neither females nor males use UV reflection for choosing their mates. Perhaps these two species were mostly found in the forests, and the spectral reflectance including UV was also relatively lower in both males and females.

Another unpredicted finding is that in the three species (*P. cavaleriei, P. vittata* and *Phintella* sp. 8), males exhibited significant differences in time spent displaying courtship behaviour to females between treatments (UV+ vs. UV–) during mate-assessment phase, which suggests that female UV reflection may be used a clue by males to make a mate choice decision in these three species. Male mate choice has attracted increasingly attention from behaviourists and it may be more common than previously thought in colourful animals showing mutual ornamentation, including birds (Hill, 1993), fishes (Houde, 1997) and butterflies (Ellers and Boggs, 2003;

Kronforst et al., 2006). However, the present study is the first evidence of UV-based male mate choice in jumping spiders. Alternatively, UV reflection may be used in sex recognition, which may require females to show its full colouration to males so that to avoid interspecies disturbance. UV-based sex recognition is widespread in animals (Guillermo-Ferreira et al., 2014; Ries et al., 2008), including jumping spider *Cosmophasis umbratica* (Lim et al., 2007b). However, further studies are needed to disentangle hypothesis about sex recognition, where we expect only the presence of UV colour (versus its absence) to be important to males, from the hypotheses about quality signalling, where we expect males to display courtship longer and more rigorously to more subtle, natural variation in the UV reflection.

The results of our study indicate that both males and females of *Phintella* species are capable of perceiving UV wavelengths and that UV is used in both male and female preferences in *P. vititta, Phintella* sp. 8, and possibly *P. cavaleriei*. The results of our study here is consistent with a previous study showing that *P. vittata* females prefer UVB+ males to UVB– males (Li et al., 2008b). However, we also showed that *P. vittata* males prefer UVB+ females to UVB– females. This is the first to show the role of UV reflection in mutual mate choice in jumping spiders.

Interestingly, *Phintella* sp. 14 females or males exhibited no significant differences between treatments due to small sample size, but there is a suggestion of possible trend for mutual mate assessment of both males and females occurring under UV– conditions. Female paid more attention to UV– males than to UV+ males, and males spent longer time to display courtship behaviour to UV– females than to UV+ females. This otherwise suggest that human-visible colours may be more important mate choice in *Phintella* sp. 14. Improved sample size may provide stronger evidence of human-visible colour based mate-choice in this species.

In conclusion, UV reflection appears to be commonly used in mate choice in tropical species of *Phintella*. We provide the first evidence of UV-based male mate-choice in some species of *Phintella* and mutual mate choice in the same species. There is a suggestion of possible trend for mutual mate assessment of both males and females occurring under UV deficient conditions, but larger sample size is needed to verify it. Although the UV blocking approach to testing the function of UV reflection is useful for determining the context in which UV colour is important, further studies are required to test whether natural variation in UV colouration in salticids mediates mate choice. This study aims to determine whether UV reflection plays a role in mate choice in salticids, but it was not designed to test whether UV reflection is more important than VIS colours in mate choice.

CHAPTER 4

The direct and indirect benefits of colour-based mating choice in the jumping spider *Chrysilla acerosa*

4.1 Introduction

Sexually dichromatism, a type of sexual dimorphism in colour in which males are bright and females are relatively dull or vice versa, is widespread across animal kingdom. The sexually dichromatic colours are often assumed to be subjected to sexual selection via female mate-choice or competition between males (Andersson, 1994; Gage et al., 2002a; Hill, 1991a; Pollux et al., 2014), natural selection (Badyaev and Hill, 2003a; Bell and Zamudio, 2012a; Owens and Hartley, 1998; Temeles et al., 2000), or the interactions between both (Badyaev and Hill, 2003a; Bell and Zamudio, 2012a; Gorman et al., 2014; Owens and Hartley, 1998). In animals, males commonly exhibit conspicuous sexual dichromatic colours, and these colours have been used to test good genes models, Fisher's runaway process, parasite theory, immunocompetence handicap, sensory exploitation, and status signalling (Andersson, 1994). Ornamental colours also occur in female (Amundsen, 2000; Amundsen et al., 1997; Tobias et al., 2012), usually as a result of sex role reversal in which males invest more resource in offspring and females compete for males (Eens and Pinxten, 2000; Kvarnemo and Ahnesjo, 1996) or from direct selection on female ornamentations (Amundsen, 2000).

However, although there is nothing uncommon in nature for both sexes to share the same ornamentations in a species (like many socially monogamous birds), the role and evolutionary mechanisms of these ornamentations usually have been neglected because overwhelmingly majority of researchers are more interested in sexually dimorphic male traits. Limited evidence has yet shown that mutual ornamentation traits usually exist in socially monogamous species in birds (Kirkpatrick et al., 1990), in which conspicuous plumage colouration is used as an

indicator of mate quality or parental investment. In addition to mate attraction associated functions, these mutual conspicuous plumage colours still can be an indicator of resource competition ability out of breeding season (Griggio et al., 2009; Pryke and Griffith, 2007; Pryke, 2007). Tobias et al. (2011) provided evidence that the mutual ornamental song in suboscine birds can help unpaired males and females to advertise for mates as well as for intrasexual resource competition. The mechanisms of the evolution of mutual ornamentations remain mysterious, but a few studies suggest that mutually shared songs in birds are connected to the level of testosterone (Kriner and Schwabl, 1991a; Velando et al., 2001a), and such sort of ornamentations is often correlated with immunocompetence, parasite load and egg mass (Martinez-Padilla et al., 2011; Vergara et al., 2011).

Almost all of the previous studies on mutual ornamentation have focused on socially paired birds, but little is known about mutual ornamentation in Arthropoda (Prudic et al., 2011) although it contains overwhelmingly majority of the total number of animal species. This probably because many investigators believe that the ornamentations are mainly for between-pair rather than within-pair interactions (Tobias et al., 2011; West-Eberhard, 1983), and their sexually function is just as a by-product. However, Prudic et al. (2011) showed that reciprocal selection through time may result in mutual sexual ornamentation in a butterfly species *Bicyclus anynana*. Therefore, mutual sexual ornamentations may be more vital and common than previously thought in arthropods.

Females often made their mating decisions based on the conspicuous colouration of courting males in creatures like birds (Gluckman, 2014; Hill, 1990, 1991a; Ibáñez et al., 2013), fish (Amundsen and Forsgren, 2001; Berglund et al., 2005; Houde, 1987), reptiles (Olsson, 1994), amphibian (Bell and Zamudio, 2012b) and

invertebrate (Clark and Uetz, 1993; Taylor and McGraw, 2013a). However, these ornament colouration traits usually not adapt to their around environments because of the high predation risks from predator or hard to access prey items (Andersson, 1994). Therefore, theoretical models predict that there must be hidden benefits associated to these ornamentations in males or in females (Eens and Pinxten, 2000) for some sex role reverse creatures. One most acceptable prediction is that the ornament colouration in males is a reflection of male quality (Amundsen, 2000; Hill, 1991a). For example, the plumage colouration in male yellowhammer, *Emberiza citronella*, is an indicator of parasite resistance, hence females mated with brighter males can produce more fledglings (Sundberg, 1995). Bonato et al. (2009) provided evidence indicating that different colours may imply different components of immune system in male ostriches (*Struthio camelus*). In addition to the correlation between ornament colouration and immune system condition, male exaggerating colouration can also be an indicator of social status in birds (Senar, 1999), fish (Parikh et al., 2006) and mammal (Setchell and Jean Wickings, 2005). In these cases, the brighter males are usually linked with a high social status and more easily to get a female. However, in most taxa, the association between male ornament colouration and female achieved benefits remains unclear (Johnstone, 1995) and overwhelmingly majority of the studies have focused on sexual dimorphic colours although there is nothing uncommon for species showing partial sexual dimorphic and partial sexual monomorphic in colour patterns.

There are two hypotheses proposed for mutual sexual shared ornamentations. The genetic correlated hypothesis (Lande, 1987; Rice, 1984) suggests that the sexually monomorphic ornaments only function in males but these characters are expressed in females merely as by-products because of the genetic correlation

between males and females. The mutual selection hypothesis (Johnstone et al., 1996; West-Eberhard, 1979) indicates that elaborate monomorphic characters may arise from selection of both sex expression in these characters. However, based on a review by Kraaijeveld et al. (2007), there is only one convincing evidence (Price (1996) that supports the genetic correlation hypothesis, and most of convincible evidence comes from mutual selection hypothesis. In this way, similar to sexual dimorphic colouration, the mutual shared colouration can be signalling in mate choice or an indicate of body conditions in intrasex competition (for both mate and food resource) (Kraaijeveld et al., 2007). The majority of studies have don on socially monomorphic birds and these studies have shown that males and females have a similar contribution in production of the mutual shared colouration (Kraaijeveld, 2003). In addition, although there is evidence that shows the mutual mate choice of elaborate colours in some animals, yet the benefits of such mutual mate choice is poorly understood. In this study, I use a jumping spider (Araneae: Salticidae) *Chrysilla acerosa* to study whether sexual dimorphic or monomorphic colouration pays a role in mutual mate choice and if so, what benefits females or males could gain.

Jumping spiders are excellent models for understanding the functions of ornamented colouration. Their charismatic and colourful courtship has intrigued biologists for years (Peckham and Peckham, 1889, 1890), yet surprisingly little empirical work has been aimed at understanding how colour may influence mating success. Many of the more than 5000 salticid species (World Spider Catalog, 2015) sport garish colouration (Jackson and Blest, 1982), and some species have strikingly iridescent markings(Li et al., 2008a; Lim and Li, 2006b; Lim et al., 2007b). Many species of salticids are sexually dichromatic (Oxford and Gillespie, 1998a). Salticids have excellent vision (De Voe, 1975a; Harland and Jackson, 2000; Land, 1969a;

Peaslee and Wilson, 1989; Yamashita and Tateda, 1976), and behavioural experiments suggest that they can discriminate between different colours (Nakamura and Yamashita, 2000; VanderSal and Hebets, 2007). Research done with the ornamented salticids, *Cosmophasis umbratica* (Lim et al., 2008b) and *Phintella vittata* (Li et al., 2008b), has shown that blocking UV light affects mate-choice decisions, suggesting that UV colouration plays an important role in courtship signalling in these species.

Chrysilla acerosa is among the most highly ornamented of jumping spiders, with a striking diversity of colourful and sexually dimorphic display traits on multiple body regions (Fig. 4.1). However, dorsal carapace, from human's visual ability, also remains sexually monochromatic. That is, both sexes have dorsal carapace exhibiting similar colours. Moreover, dorsal carapace appears to be much brightly coloured compared to other body regions (dorsal abdomen, lateral abdomen and lateral carapace) in both males and females. *C. acerosa* is thus an ideal model to investigate whether the presence of sexually dichromatic or monochromatic ornamentation is required for or improving successful mating. First, I quantified the colour of four main body regions (dorsal abdomen, lateral abdomen, dorsal carapace and lateral carapace) of males and females using spectrophotometer, and tested for the existence of sexual dichromatism and monochromatism, including UV reflection (wavelength < 400 nm). After I found that three body regions (dorsal abdomen, lateral abdomen, and lateral carapace) were sexually dichromatic in the UV, human-visible (VIS: 400–700 nm) wavelength range, or both, whereas the dorsal carapace was sexually monochromatic in both the UV and VIS ranges. I then went on to conduct mating experiments by pairing individual males and virgin females under full-spectral lighting to test whether the presence of sexually dichromatic or monochromatic colour

of a specific body region is either required for or improving successful mating in *C. acerosa*. Moreover, I investigated the benefits gained from successful mating based on sexually monomorphic colouration in male dorsal carapace. Simultaneously, the effects of sexual dimorphic colouration on each mentioned stages have been analysed as well. To our knowledge, this is the first investigation which analysed both the sexual monomorphic and dimorphic colour linked benefits simultaneously.

4.2 Materials and methods

4.2.1 Study subjects and maintenance

Adult *Chrysilla acerosa* males and females both exhibit bright body colouration, but it shows obvious sexual dichromatism in some body regions (**Figure 4.1**). *Chrysilla acerosa* were collected as large juveniles and sub-adults (*N* = 257) from dry bamboos in Nanchong (30.507 N, 106.036 E. 206 m a.s.l.), Sichuan, China in January 2013. They were then transported to the laboratory at the National University of Singapore (Singapore), kept individually in plastic cylindrical cages (diameter x height: 60 mm x 80 mm) and maintained in the laboratory with controlled environmental conditions (relative humidity: 80-90%; temperature: 25 ± 1℃; 12:12 h light/dark cycle; lights on 08:00 h). Fruit flies (*Drosophila melanogaster*) were provided to them twice a week as food items. All spiders were monitored once every two days until they reached sexual maturation (i.e. the last moult) so that their post-maturation age was known. All spiders were virgins, which allowed us to eliminate the possibility of previous sexual encounters with conspecifics.

4.2.2 Sexual dichromatism quantification

Spectral reflectance was measured on the 10th day after maturity for 20 virgin males and 20 virgin females, respectively, following the standard protocols (Cuthill et al., 1999; Endler and Thery, 1996; Lim and Li, 2006b). Four body regions were selected for spectral reflectance measurements based on display behaviour as described in early salticid studies (Li et al., 2008a; Li et al., 2008c; Lim and Li, 2006b). The four body regions include dorsal abdomen (DA), dorsal carapace (DC), lateral abdomen (LA) and lateral carapace (LC). To collect the spectra reflectance data, I used an

Ocean Optics USB4000 spectrometer (Ocean Optics Inc.) and a DH2000 deuterium & tungsten halogen light source (Ocean Optics Inc., Dunedin, USA). The individual spider was anaesthetized with CO_2 and then mounted on a fixed stage for spectra

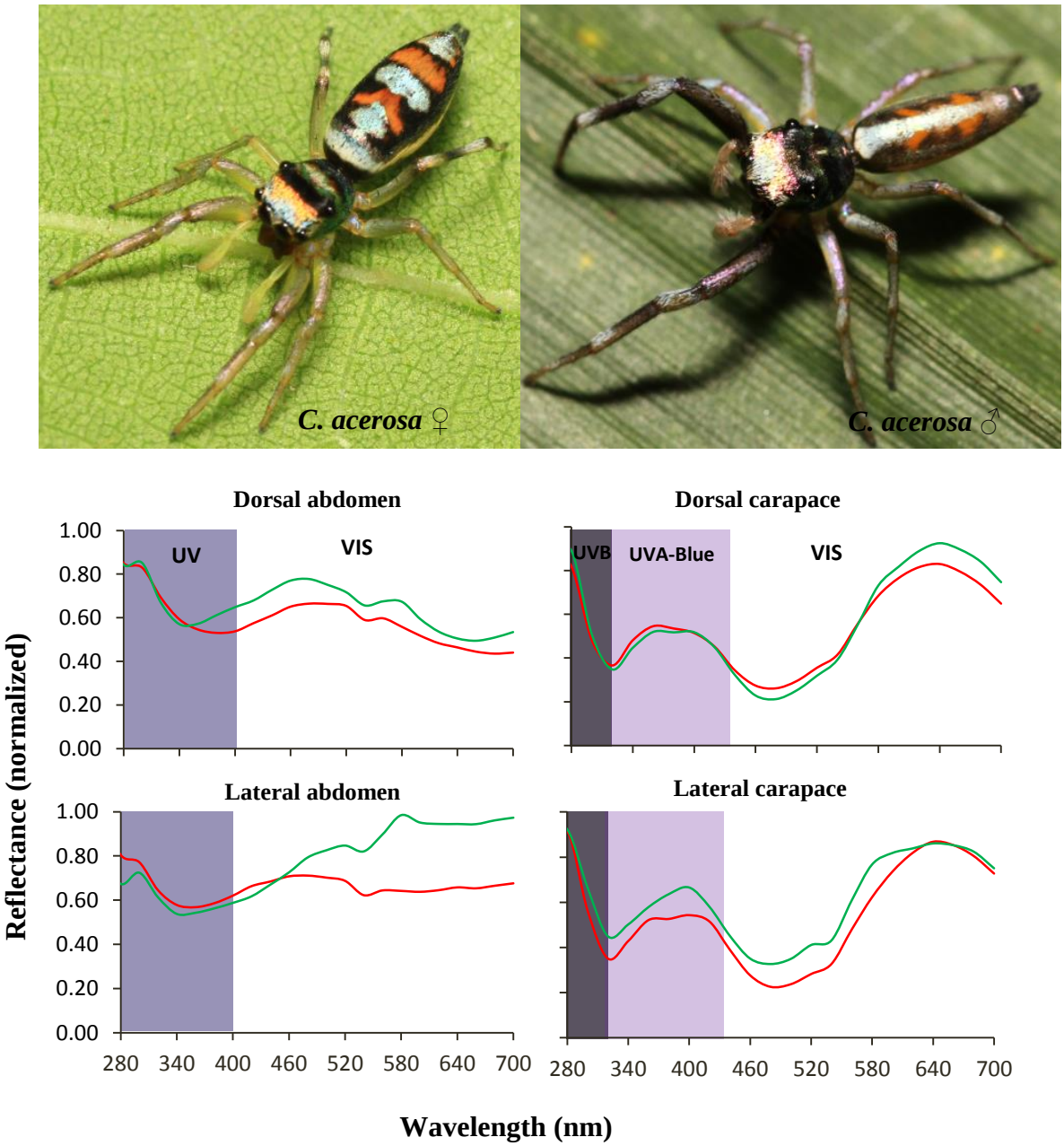


Figure 4.1. Male and female *Chrysila acerosa* as well as sexual dichromatism in four body regions of dorsal carapace, dorsal abdomen, lateral abdomen and lateral carapace (green line: female, red line: male). UV: wavelength 280-400 nm; VIS: wavelength: 400-700 nm for dorsal abdomen and lateral abdomen; and 450-700 nm for dorsal carapace and lateral carapace wavelength; UVB: wavelength 280-320 nm; UVA-Blue Wavelength: 320-450 nm

reflectance measuring. The reflectance probe was kept consistently at 2 mm from spider's body surface and at a right angle from the measurement surface. Details of the method are consistent with a previous study (Lim and Li, 2006b). Five reflectance spectra were measured from each specific body region of each individual. These spanned from UV to VIS (human visible) wavelength ranges (280–700 nm). Spectra were taken at 1 nm intervals for each of the original reflectance spectrum (spectra range: 280–700 nm). The spectra were then normalized as every data divided by the maximum reflectance value, and in this way, all the reflectance data were transformed to a value from 0 to 1.

4.2.3 Mating success experiment

To determine the effects of naturally occurring variation in male display colouration on male mating success, I randomly chose one male and one female about 15-20 days after they reached maturity and introduced them into a glass chamber (L × B × H = 7.5 cm ×7.5 cm ×7.5 cm). The inner sides and bottom of the chamber were covered by a sheet of white paper, and the top of the chamber was covered by a quartz glass which transmitted full spectral light. The illumination was provided by 10 Voltarc Ultra tubes (110 W each, powered by a 120 V50/60 Hz electronic ballast; SUPER-TEK, Naturallighting.com, Houston, Texas) and 2 additional UV light emitting dark tubes (Hitachi BL/B, 20W each, powered by a 230 V 50/60 Hzelectronic ballast), all of which were suspended about 150 cm above the chamber. All of the light tubes and mating chamber were fully surrounded by black cloth to minimize the observer's interference. For each mating trial, the pair was give 15 min for mating and I videotaped all interactions. At the conclusion of each mating trial, I then removed the male and the female back their original cages. Between trials, I replaced a new sheet of white paper, and used 70% ethanol to clean the chamber and the top covered quartz

glass. A total of 82 mating trials were conducted. For the fail mated trails, repeated mating experiments have been conducted among the individuals with randomly paired the male and female individuals. This section has been down in purpose of increase the sample size of offspring.

I then measured the spectral reflectance of all the males that had successfully mated and failed to mate using the same protocol as described above. Just after each trial ended, I weighed males to the nearest 0.0001 g with a digital scale in order to determine the effect of male body size on mating success. By playing back the videos, I recorded courtship latency (the interval between the start of courtship display and copulation) and copulation duration (the interval between the start and the end of copulation).

4.2.4 Male colour and the benefits

To determine whether females gained direct or indirect benefits by successfully mating with males they chosen, I examined a number of fitness components. In doing so, I returned all the successful mated females to their previous maintenance containers accordingly and used the same rearing method as mentioned above until they naturally died. I monitored females daily for egg laying, and if they produced eggs, then checked for egg hatching, number of hatchlings, and number of unhatched eggs. The hatchlings were then separated from their mother one week later and housed individually using the protocol as described above. Spiderlings were fed with honey and small fruit fly (*Drosophila melanogaster*) larva simultaneously twice a week in the first four weeks after they were separated from their mother, and then they were provided adult fruit flies twice a week. I checked daily for survivorship of juveniles.

4.2.5 Data analysis

4.2.5.1 Sexual dichromatism qualification

As there are one reflectance peak at UV (wavelength: 280-400 nm) and the other peak at VIS (wavelength: 400-700 nm) range, respectively, on the dorsal and lateral abdomen **(Fig. 4.1)**, I analysed two reflectance ranges, UV and VIS for these two body regions. Three reflectance peaks present at UVB, UVA-Blue and VIS on the dorsal and lateral carapace (**Fig. 4.1**), hence I analysed UVB (wavelength: 280-320 nm), UVA-Blue (wavelength: 320-450 nm) and VIS (wavelength: 450-700 nm) for these two body regions. I used three standard descriptions of reflectance spectra as used in the previous studies (Li et al., 2008a; Lim and Li, 2006b) namely total brightness (spectral intensity or percent reflectance (%) of the reflection band, R), hue (spectral location or wavelength (nm) of maximal reflectance, λ_{max}), and maxmin-chroma (saturation or spectral purity)). To determine the presence/absence of sexual dichromatism, AVICOL V.6 (GOMEZ, 2006) was used to calculate total brightness (B), hue (H) and maxmin-chroma (C) for each reflectance range of each body region. I then performed repeated-measures multivariate analysis of variance (MANOVA) to test for the effects of sex and body regions as well as their interaction on overall colouration (i.e., taking consideration of three colour metrics (B, H and C) into account). Individual colour metrics were then analysed for each body region by one-way repeated-measures ANOVAs.

4.2.5.2 Male colour and mating success

I then used binary logistic regression backward model to test for the effects of male colouration (three colour metrics of each wavelength range for each body region as

predictors), body weight, male and female post-maturation age on male mating success (dependent variable: mated or failed to mate). As male body weight, male and female post-maturation age were not good predictors for mating success in the initial model, I removed these three predictors from the final model. I then compared coloration differences between the males that successfully mated and the males that failed to mate for each of the four body regions (DA, LA, DC and LC) using MANOVA and one-way repeated-measures ANOVAs. As males showed significant differences only in the DC spectral reflectance, I compared the difference in the DC colouration between females and the mated males as well as between females and the males that failed to mate separately using one-way repeated-measures ANOVAs. In these comparisons, female colouration data were those used for analysis of between-sex colouration variations above. All the data were analysed using SPSS, version 22.0 (IBM SPSS). Two-tailed tests were employed.

4.2.5.3 Male colour and the benefits

I analysed the data on courtship latency and copulation duration based on males that mated successfully in the first mating trials only, while I analysed the data on other fitness components based on the males that mated successfully in the first mating trials and in second mating trials. I used binary logistic (LOG) models analysed the effects of male colouration on the egg production (whether the mated female produced eggs), egg hatching (whether eggs hatched) and number of egg-sacs (whether the females produced only 1 egg-sac or 2 egg-sacs) with. I then used linear regression (LIN) models (backward) to analyse the effects of male colouration on courtship latency, copulation duration, the number of eggs of egg sac 1, hatching rate of eggs (the number of hatched spiderlings of the first egg-sac / the number of eggs of

the first egg -sac), the total number of eggs (sum of egg number from all egg-sacs produced by each female), the total number of hatchlings (the number of spiderlings from all egg-sacs produced by each female), total hatching rate (total hatchings number / total egg number of each female), and juvenile survivorship (the number of juveniles survived until sub-adults). For the survivorship, I analysed the effects of abdomen (including dorsal abdomen and lateral abdomen) colouration and carapace (including dorsal carapace and lateral carapace) colouration on the survivorship. All the data analyses were performed using SPSS version 22.0.

4.3 Results

4.3.1 Sexual dichromatism

Results from MANOVA revealed a significant effect of sex, body region and the interaction between sex and body region on both abdomen and carapace colouration (**Table 4.1**). Both dorsal and lateral abdomens were sexually dichromatic in all the three colour components (Qt, hue and chroma) of both the UV and VIS ranges except for dorsal abdomen UV hue and UV chroma as well as dorsal abdomen VIS chroma (**Table 4.2**). For the carapace, lateral carapace was sexually dichromatic in the Qt and chroma, but not in the hue, of all three wavelength ranges (UVB, UVA-Blue and VIS), but dorsal carapace was not sexually dichromatic in any colour component of any of three wavelength ranges (**Table 4.3**).

Table 4.1. Results from repeated-measures MANOVAs testing for the effects of sex, body region and their interaction on the spectral reflectance of body regions (abdomen: dorsal abdomen and lateral abdomen; carapace: dorsal carapace and lateral carapace).

		Wilks' λ	F	P
Abdomen	Sex	0.348	$F_{6,71} = 22.164$	< 0.001
	Abdomen	0.157	$F_{6,71} = 63.647$	< 0.001
	Sex × abdomen	0.544	$F_{6,71} = 9.900$	< 0.001
Carapace	Sex	0.569	$F_{9,68} = 5.734$	< 0.001
	Carapace	0.514	$F_{9,68} = 7.151$	< 0.001
	Sex × carapace	0.666	$F_{9,68} = 3.784$	< 0.001

Table 4.2. Results from repeated-measures ANOVAs testing for between-sex differences in UV and VIS colouration of dorsal abdomen (DA) and lateral abdomen (LA). Qt: total brightness.

		UV		VIS	
Body region	Colour metrics	$F_{1,76}$	*P*	$F_{1,76}$	*P*
DA	Qt	4.844	0.034	19.422	< 0.0001
	Hue	0.449	0.507	5.071	0.03
	Chroma	3.242	0.08	0.607	0.441
LA	Qt	8.619	0.006	39.193	0.000
	Hue	7.523	0.009	5.033	0.031
	Chroma	39.193	< 0.0001	12.951	0.001

Table 4.3. Results from repeated-measures ANOVAs testing for between-sex differences in UVB, UVA-Blue and VIS colouration of dorsal carapace (DC) and lateral carapace (LC).

		UVB		UVA-Blue		VIS	
Body region	Colour metrics	$F_{1,38}$	*P*	$F_{1,38}$	*P*	$F_{1,38}$	*P*
DC	Qt	0.676	0.416	0.736	0.396	1.414	0.242
	Hue	1.572	0.218	0.351	0.557	2.078	0.158
	Chroma	0.076	0.784	1.284	0.264	0.718	0.402
LC	Qt	17.939	0.000	29.88	0.000	18.144	0.000
	Hue	1.112	0.298	1.83	0.184	3.921	0.055
	Chroma	19.749	< 0.0001	6.575	0.014	25.727	< 0.0001

4.3.2. Male colouration and mate success

Forty six out of 82 males (56.1%) copulated during the first mating trials. As at least one colour component of at least one body region were unable to calculate for four out of 46 mated males and for one out of 36 males that had failed to mate, I excluded these five males from data analyses. Results from binary logistic regression showed that male dorsal carapace VIS chroma, lateral abdomen VIS hue, lateral abdomen VIS chroma and lateral carapace VIS total brightness might predict successful mating, but only dorsal carapace VIS chroma significantly predicted successful mating (**Table 4.4**): males that have a higher dorsal carapace VIS chroma were more likely to successfully mate.

Table 4.4. The results from a Wald backward binary logistic regression testing for the effects of every colour component (Qt, hue and chroma) of each wavelength range for each of four male body regions on mating success (likelihood of copulation; response variable). DC: dorsal carapace; LA: lateral abdomen; LC: lateral carapace; VIS: 450-700 nm for DC and LC; VIS: 400-700 nm for LA; Qt: total brightness. Hosmer & Lemeshow *R2* = 0.14; Cox & Snell R^2 = 0.18, Nagelkerke R^2 = 0.24; Omnibus test of model coefficients: χ_4^2= 15.326, p = 0.004.

Body region	Reflectance range	Colour metrics	B (S.E.)	Wald	*df*	P	95% CI for odds ratio		
							Lower	Odds ratio	*Upper*
DC	VIS	Chroma	1.91 (0.71)	7.363	1	0.007	1.70	6.78	27.01
LA	VIS	Hue	-0.01 (0.003)	2.734	1	0.098	0.99	0.995	1.00
LA	VIS	Chroma	3.71 (2.06)	3.254	1	0.071	0.73	40.98	2315.6
LC	VIS	Qt	0.03 (0.02)	3.304	1	0.069	1.00	1.03	1.06
		Constant	-4.88 (2.89)	2.862	1	0.091		0.01	0.091

Results from One-way ANOVAs revealed significant differences only in the dorsal carapace UVB Qt, UVB chroma, UVA-Blue Qt and VIS chroma, but not in any other colour components of dorsal carapace and other three body regions between the successfully mated males and the males that did not mate in the first mating trials (**Table 4.5**). I then compared the differences in dorsal carapace colours between females and successfully mated males as well as between females and males that failed to mate. The results from ANOVAs showed that the successfully mated males had similar dorsal carapace UVB hue and VIS chroma that well matched the colours of females with which the males mated. However, males that had failed to copulate had significant different colours on dorsal carapace UVB hue and VIS chroma from females **(Table 4.6; Fig. 4.2)**.

Table 4.5. The differences in spectral reflectance between males that had successfully mated and males that had failed to mate. DA: dorsal abdomen, LA: lateral abdomen, DC: dorsal carapace, LC: lateral carapace. UVB: 280-320 nm, UV: 280-400 nm, UVA-Blue: 320-450 nm, VIS: 400-700 nm for DA and LA, VIS: 450-700 nm for DC and LC, B: total brightness, C: Maxmin-Chroma. Mated males: N = 46, males that failed to mate: N = 36.

Body Region	Reflectance range	Colour metrics	Mate success	Mean	S.E.	*F*	*p*
DA	UV	B	Mated	82.10	1.25	1.57	0.214
			Failed	79.62	1.57		
DA	UV	H	Mated	291.48	1.25	0.23	0.632
			Failed	290.67	1.05		
DA	UV	C	Mated	0.59	0.02	1.32	0.254
			Failed	0.63	0.03		
DA	VIS	B	Mated	179.07	4.52	1.08	0.302
			Failed	171.86	5.32		
DA	VIS	H	Mated	497.87	5.71	0.39	0.535
			Failed	504.42	9.39		
DA	VIS	C	Mated	0.54	0.02	2.24	0.138
			Failed	0.60	0.04		
DC	UVB	B	Mated	21.13	0.69	6.96	0.010**
			Failed	24.07	0.90		
DC	UVB	H	Mated	280.78	0.43	3.31	0.073
			Failed	282.43	0.87		
DC	UVB	C	Mated	1.01	0.06	5.23	0.025*
			Failed	0.79	0.08		
DC	UVA-Blue	B	Mated	58.21	1.38	4.66	0.034*
			Failed	63.39	2.07		
DC	UVA-Blue	H	Mated	369.82	3.53	1.73	0.193
			Failed	361.97	5.03		
DC	UVA-Blue	C	Mated	0.77	0.04	3.61	0.061
			Failed	0.64	0.05		
DC	VIS	B	Mated	140.90	3.17	0.4	0.53
			Failed	143.78	3.19		
DC	VIS	H	Mated	636.36	4.71	2.88	0.094
			Failed	620.83	8.43		
DC	VIS	C	Mated	1.27	0.05	7.44	0.008**
			Failed	1.02	0.08		
LA	UV	B	Mated	79.07	1.28	0.01	0.937
			Failed	79.24	1.68		
LA	UV	H	Mated	294.47	3.35	1.01	0.318
			Failed	290.64	1.12		
LA	UV	C	Mated	0.42	0.02	0.19	0.668
			Failed	0.44	0.02		

LA	VIS	B	Mated	207.66	5.99	0.18	0.676
			Failed	211.39	6.57		
LA	VIS	H	Mated	551.23	14.32	1.84	0.179
			Failed	580.28	16.01		
LA	VIS	C	Mated	0.49	0.03	2.64	0.108
			Failed	0.43	0.02		
LC	UVB	B	Mated	23.76	0.68	0.17	0.682
			Failed	24.15	0.65		
LC	UVB	H	Mated	280.51	0.23	0.24	0.623
			Failed	280.72	0.38		
LC	UVB	C	Mated	0.97	0.03	0.32	0.574
			Failed	1.01	0.05		
LC	UVA-Blue	B	Mated	60.67	1.27	0.35	0.558
			Failed	59.57	1.37		
LC	UVA-Blue	H	Mated	378.93	3.98	0.01	0.932
			Failed	379.44	4.48		
LC	UVA-Blue	C	Mated	0.66	0.03	0.00	0.981
			Failed	0.66	0.04		
LC	VIS	B	Mated	136.14	2.69	0.25	0.617
			Failed	134.14	2.91		
LC	VIS	H	Mated	643.16	2.68	0.90	0.347
			Failed	647.39	3.72		
LC	VIS	C	Mated	1.26	0.03	0.17	0.681
			Failed	1.28	0.04		

Table 4.6. Comparisons of dorsal carapace spectral reflectance between females and successfully mated males as well as between females and males that had failed to mate. Female: $N = 20$, mated male $N = 45$, males that failed to mate: $N = 35$.

Reflectance range	Colour metrics	Sex & mating success	Mean	S.E	*F*	P
		Female	22.73	0.87		
UVB	B	Mated male	21.13	0.69	1.820	0.182
		Male failed to mate	24.07	0.90	0.965	0.330
		Female	280.00	0.00		
UVB	H	Mated male	280.78	0.43	1.419	0.238
		Male failed to mate	282.43	0.87	4.457	0.039
		Female	1.01	0.05		
UVB	C	Mated male	1.01	0.06	0.00	0.984
		Male failed to mate	0.79	0.08	3.995	0.051
		Female	59.19	1.10		
UVA-Blue	B	Mated male	58.21	1.38	0.200	0.656
		Male failed to mate	63.39	2.07	2.139	0.149
		Female	373.05	4.39		
UVA-Blue	H	Mated male	369.82	3.53	0.284	0.596
		Male failed to mate	361.97	5.03	2.209	0.143
		Female	0.62	0.03		
UVA-Blue	C	Mated male	0.77	0.04	4.386	0.040
		Male failed to mate	0.64	0.05	0.031	0.861
		Female	149.14	1.94		
VIS	B	Mated male	140.90	3.17	2.771	0.101
		Male failed to mate	143.78	3.19	1.429	0.237
		Female	635.80	2.49		
VIS	H	Mated male	636.36	4.71	0.006	0.939
		Male failed to mate	620.83	8.43	1.737	0.193
		Female	1.23	0.03		
VIS	C	Mated male	1.27	0.05	0.216	0.644
		Male failed to mate	1.02	0.08	4.051	0.049

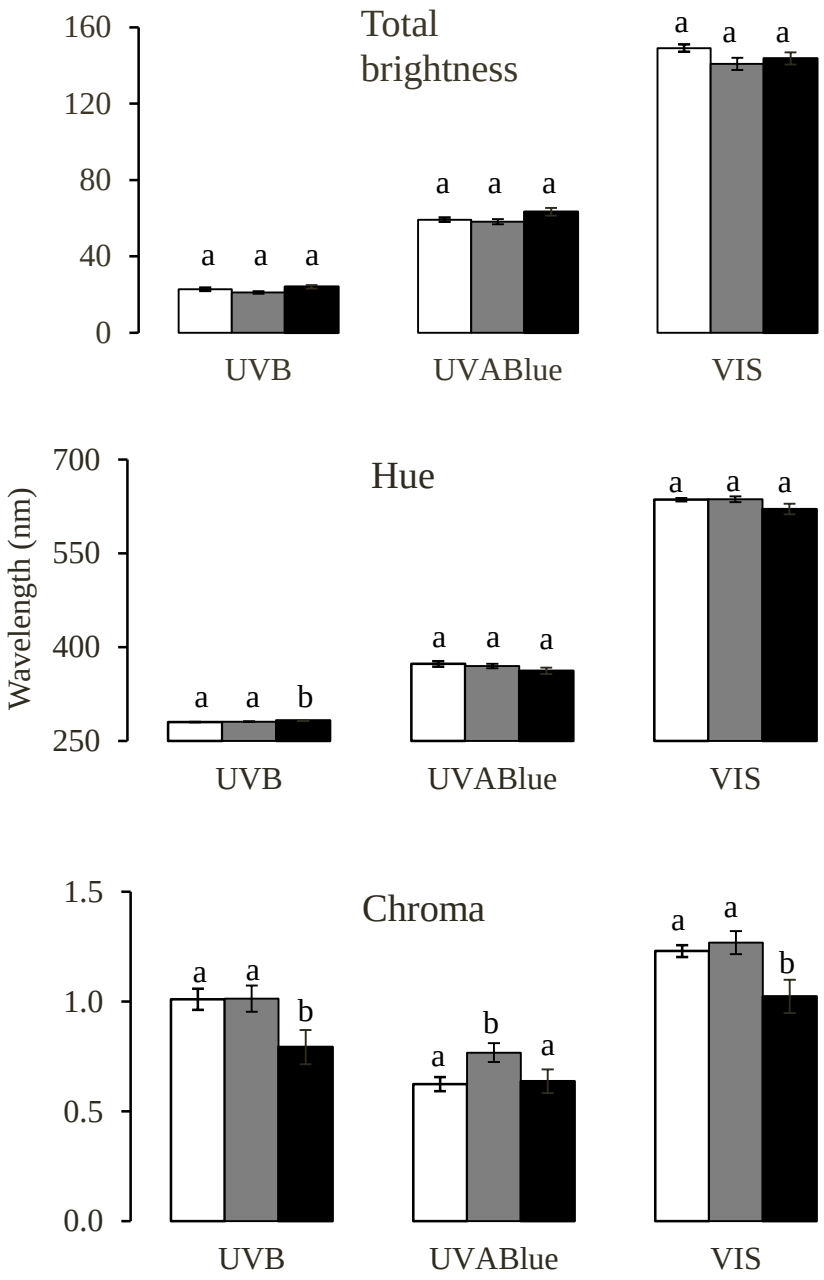


Figure 4.2. The differences in the total brightness (Qt), hue (nm) and chroma of *Chrysila acerosa* dorsal carapace of females (*N* = 20; white bar), successfully mated males (*N* = 45; grey bar) and males (*N* = 35; black bar) that failed to mate. Different lower letters indicate significant difference.

4.3.3 Male colour and benefits

4.3.3.1 Mating, egg production, hatching and offspring survivorship

The mean courtship latency and copulation duration were 368.5 (± 32.7 (SE)) s and 666.4 (± 73.1) s (N = 47), respectively. Among the males that failed to mate in the first mating trials, 20 successfully mated in the second mating trials. Forty two out of the 67 mated females successfully produced eggs, 18 of which produced two egg-sacs and one of the 18 females produced three egg-sacs. Among the first egg-sacs, 22 out of the 42 egg-sacs successfully hatched, and the mean hatching duration were 26.3 (± 1.0) days. The mean number of eggs of the first egg-sac were 14 (± 1) (N = 42) and the mean number of hatchlings was 5 (± 1) (N = 42). Among the second egg-sacs, the mean number of eggs per egg-sac were 11 (± 1) (N = 18), and the number of hatchlings were 1.6 (± 0.9) (N =18). The mean hatching rate of the first egg-sacs was 37 (± 7) % (N = 37) and 14 (± 7) % (N = 18) for the second egg-sacs. On average, the hatching rate of eggs was 31 (± 7) % (N = 42, SE = 0.06). The mean number of 7.6 (± 0.9) spiderlings (N = 23) survived over the first week (until separated from their mother) for each female. The juveniles from 19 females survived until sub-adults, and the mean number of juveniles per females that survived until sub-adults were 4.4 (± 0.6) (N = 19).

4.3.3.2 Courtship latency and copulation duration

Linear regressions showed that colourations of all the four body regions had a significant effect on courtship latency (R = 0.688, $F_{10, 31}$ = 2.782, P = 0.014) (**Table 4.7**). The colouration of dorsal carapace, lateral carapace and lateral abdomen had significant effects on copulation duration but dorsal abdomen colouration had no significant effect on copulation duration (R = 0.725, $F_{9, 32}$ = 3.947, P = 0.002). VIS

chroma positively affected courtship latency and copulation duration. In details, VIS chroma of dorsal abdomen (DA), dorsal carapace (DC) and lateral carapace (LC) positively affected courtship latency. However, UV colouration had negative effects on courtship latency. Chroma had a significant positive effect on copulation duration, but the sexual dimorphic VIS and UV chroma of lateral abdomen negatively affected copulation duration. In contrast, the sexual monomorphic UVA-Blue chroma of DC positively affected copulation duration. In addition, the total brightness of UV and VIS of lateral abdomen (LA) positively and negatively affected copulation duration, respectively.

4.3.3.3 Egg production, egg hatching and egg sac number

Results from binary logistic regression revealed that male sexual monomorphic (SM) colour of dorsal carapace was a better predictor of probability of egg production (Wald = 3.013, Omnibus Test χ_4^2 = 12.377, P = 0.015; **Table 4.8**). Total UVB brightness, UVB chroma, total UVA-Blue brightness and UVA-Blue hue and VIS hue of male dorsal carapace were better predictor of whether females would produce an egg-sac or not. Both SM and sexual dimorphic (SD) colours of males could predictor whether eggs were unable to hatch or not. The eggs produced by the females that had mated males with SM colour of male dorsal carapace, SD of dorsal abdomen and lateral carapace were less likely to hatch **(Table 4.8)**. Specifically, the eggs produced by females that had mated the males with bright UVA-Blue dorsal carapace and longer UVB-Blue and VIS hue were more likely to hatch, but the eggs produced by the females that had mated the males with higher VIS chroma dorsal abdomen and brighter UVA-Blue lateral carapace were less likely to hatch. Male colouration of all

the four body regions had considerable effects on the number of egg-sacs, but the main effects came from SD colours of dorsal and lateral carapace (**Table 4.8**).

Table 4.7. Results from binoary logistic regressions testing the effects of male colouration effects on egg production, egg hatching and number of egg-sac. The colouration of abdomen (including dorsal abdomen and lateral abdomen) and carapace (including dorsal carapace and lateral carapace) was analysed respectively. DA: dorsal abdomen, LA: lateral abdomen, DC: dorsal carapace, LC: lateral carapace, B: total brightness, H: Hue, C: chroma. Cons = constant.

	Body region	Reflectance Range	PCA	B	S.E.	Wald	df	Exp (B)	P
Egg production	LA	UV	H	-0.047	0.032	2.069	1	0.954	0.15
	Cons.			14.151	9.43	2.252	1	1.40E+06	0.133
	DC	UVB	B	-0.258	0.104	6.204	1	0.773	0.013
	DC	UVA-Blue	B	0.19	0.073	6.806	1	1.209	0.009
	DC	VIS	H	0.023	0.012	3.925	1	1.024	0.048
	DC	UVB	C	3.552	1.508	5.547	1	34.898	0.019
	Cons.			-24.081	10.69	5.074	1	0	0.024
	DA	VIS	C	-5.655	2.744	4.247	1	0.003	0.039
	Cons.			3.295	1.547	4.534	1	26.969	0.033
Egg hatching	DC	UVB	B	-0.65	0.333	3.818	1	0.522	0.051
	DC	UVA-Blue	B	0.778	0.329	5.586	1	2.178	0.018
	DC	UVA-Blue	H	0.066	0.029	5.159	1	1.069	0.023
	DC	VIS	B	-0.175	0.078	5	1	0.839	0.025
	DC	VIS	H	0.065	0.033	3.871	1	1.068	0.049
	LC	UVA-Blue	B	-0.188	0.088	4.539	1	0.829	0.033
	Cons.			-67.092	27.405	5.993	1	0	0.014

	DA	UV	B	0.573	0.268	4.582	1	1.774	0.032
	DA	UV	C	-15.919	6.927	5.281	1	0	0.022
	DA	VIS	B	-0.222	0.091	5.882	1	0.801	0.015
	DA	UV	H	0.066	0.028	5.766	1	1.068	0.016
	LA	VIS	H	-0.552	0.228	5.863	1	0.576	0.015
	LA	UV	C	-30.118	13.501	4.976	1	0	0.026
	LA	VIS	H	0.018	0.01	3.566	1	1.019	0.059
	Cons.			131.01	52.61	6.201	1	7.89E+56	0.013
Egg-sac 1 or 2									
	DC	UVB	C	3.151	1.833	2.956	1	23.366	0.086
	DC	UVA-Blue	C	-8.642	3.746	5.322	1	0	0.021
	LC	UVB	C	-10.838	4.362	6.175	1	0	0.013
	LC	UVA-Blue	H	-0.054	0.027	3.994	1	0.947	0.046
	LC	UVA-Blue	C	8.245	3.804	4.698	1	3.81E+03	0.03
	LC	VIS	C	7.359	3.576	4.234	1	1.57E+03	0.04
	Cons.			21.462	11.926	3.238	1	2.09E+09	0.072

Table 4.8. Results from linear regression models testing the effects of male colouration on courtship latency, copulation duration, the number of eggs of egg-sac 1, hatching rate of egg-sac 1, total number of eggs, total number of hatchlings, total hatching rate, survivorship 1 (spiderlings survived till one week) and survivorship 2 (spiderlings survived till sub-adult). DA: dorsal abdomen, LA: lateral abdomen, DC: dorsal carapace, LC: lateral carapace, B: total brightness, H: Hue, C: chroma.

	Body region	**Reflectance range**	**PCA**	**B**	**S.E.**	**β**	**t**	***P***
Courtship latency	Cons.			16092.85	4809		3.346	0.002
	DA	UV	B	-19.819	8.86	-0.558	-2.237	0.033
	DA	VIS	B	4.199	2.4	0.459	1.751	0.09
	DA	VIS	C	966.275	265	0.53	3.649	0.001
	DC	UVB	B	20.158	10.1	0.42	2.002	0.054
	DC	UVB	H	-62.917	18.4	-0.841	-3.411	0.002
	DC	UVA-Blue	B	21.553	10.7	0.899	2.013	0.053
	DC	UVA-Blue	H	-3.46	1.85	-0.355	-1.868	0.071
	DC	VIS	C	593.321	263	0.951	2.257	0.031
	LA	VIS	H	0.905	0.35	0.382	2.604	0.014
	LC	VIS	C	517.779	179	0.462	2.886	0.007
Copulation duration	Cons.			-3514.95	2959		-1.188	0.244
	DC	UVB	C	-430.399	216	-0.371	-1.989	0.055
	DC	UVA-Blue	C	721.653	322	0.467	2.241	0.032
	LA	UV	B	34.438	11.9	0.645	2.889	0.007
	LA	UV	C	-3060.69	848	-0.673	-3.61	0.001
	LA	VIS	B	-17.05	3.48	-1.487	-4.907	<0.001
	LA	VIS	C	-1175.14	333	-0.565	-3.529	0.001
	LC	UVB	B	-79.493	19.5	-0.816	-4.075	<0.001
	LC	UVA-Blue	H	5.295	2.44	0.313	2.166	0.038
	LC	VIS	H	10.251	4.53	0.391	2.264	0.03

	Cons.			-817.216	158.609		-5.152	<0.001
	DA	VIS	C	-16.681	4.063	-0.482	-4.105	<0.001
	DC	UVB	H	0.639	0.176	0.504	3.636	0.001
	DC	UVA-Blue	B	-0.461	0.134	-0.873	-3.437	0.002
	DC	VIS	C	-10.445	2.522	-0.832	-4.141	0
	LA	UV	B	0.371	0.108	0.634	3.434	0.002
No of eggs of egg-sac 1	LA	VIS	H	-0.079	0.026	-0.615	-3.036	0.005
	LC	UVB	H	2.135	0.43	0.595	4.971	<0.001
	LC	UVB	C	14.433	2.755	0.738	5.238	<0.001
	LC	UVA-Blue	H	0.049	0.024	0.254	2.015	0.054
	LC	VIS	B	0.216	0.048	0.791	4.45	<0.001
	LC	VIS	H	-0.127	0.025	-0.531	-4.977	<0.001
	Cons.			-17.18	3.302		-5.203	<0.001
	DA	UV	B	0.038	0.012	0.62	3.179	0.004
	DA	UV	C	2.315	0.574	0.661	4.031	<0.001
	DA	VIS	C	-1.727	0.478	-0.559	-3.61	0.001
	DC	UVB	B	0.034	0.014	0.353	2.389	0.025
	DC	UVA-Blue	H	0.013	0.003	0.742	3.828	0.001
	DC	UVA-Blue	C	0.903	0.413	0.559	2.186	0.039
Hatching rate of egg-sac1	DC	VIS	B	-0.007	0.004	-0.37	-1.97	0.06
	LA	UV	C	-1.107	0.316	-0.989	-3.505	0.002
	LA	VIS	B	-0.018	0.009	-0.34	-1.947	0.063
	LA	VIS	H	-0.002	0.001	-0.377	-2.706	0.012
	LC	UVB	B	-0.047	0.017	-0.432	-2.77	0.011
	LC	UVA-Blue	H	0.009	0.003	0.55	3.477	0.002
	LC	VIS	H	0.013	0.003	0.632	4.111	<0.001
	Cons.			-699.605	277.513		-2.521	0.019
Total no. of eggs	DA	UV	H	-0.364	0.151	-0.424	-2.417	0.024

	DA	VIS	B	-0.129	0.069	-0.458	-1.857	0.076
	DA	VIS	H	0.117	0.047	0.537	2.466	0.021
	DA	VIS	C	-24.928	7.032	-0.444	-3.545	0.002
	DC	VIS	B	-0.135	0.07	-0.373	-1.918	0.067
	DC	VIS	C	-11.168	3.185	-0.549	-3.507	0.002
	LA	UV	B	0.603	0.229	0.637	2.636	0.014
	LA	UV	C	-23.978	8.687	-0.447	-2.76	0.011
	LA	VIS	B	-0.202	0.062	-0.974	-3.24	0.003
	LC	UVB	H	2.827	0.898	0.486	3.146	0.004
	LC	UVB	C	33.072	6.75	1.044	4.899	<0.001
	LC	VIS	B	0.433	0.11	0.98	3.939	0.001
	LC	VIS	H	-0.231	0.056	-0.599	-4.11	<0.001
	Cons.			-85.226	24.01		-3.55	0.001
Total no. of hatchlings	DA	UV	B	0.346	0.16	0.355	2.162	0.039
	DC	UVA-Blue	H	0.149	0.052	0.529	2.884	0.007
	DC	VIS	H	-0.144	0.065	-0.458	-2.235	0.033
	DC	VIS	C	-8.678	3.568	-0.49	-2.432	0.021
	LA	VIS	H	-0.025	0.01	-0.346	-2.625	0.013
	LC	UVA-Blue	B	-0.514	0.136	-0.584	-3.783	0.001
	LC	UVA-Blue	H	0.189	0.049	0.693	3.821	0.001
	Cons.			29.247	14.65		1.996	0.057
	DA	UV	H	-0.015	0.007	-0.36	-1.948	0.063
	DA	VIS	B	0.01	0.003	0.717	2.911	0.008
	DC	UVB	B	0.113	0.039	1.356	2.899	0.008
Total hatching rate	DC	UVB	H	-0.093	0.04	-0.962	-2.333	0.028
	DC	UVA-Blue	H	0.014	0.003	0.889	4.152	<0.001
	DC	UVA-Blue	C	0.736	0.389	0.531	1.894	0.07
	DC	VIS	H	-0.01	0.003	-0.913	-2.893	0.008

	LA	UV	B	-0.041	0.014	-0.909	-2.872	0.008
	LA	VIS	C	-0.76	0.381	-0.373	-1.996	0.057
	LC	UVB	B	-0.094	0.033	-1.019	-2.87	0.008
	LC	VIS	B	-0.01	0.004	-0.47	-2.234	0.035
	LC	VIS	H	0.014	0.005	0.791	2.961	0.007
	LC	VIS	C	-0.799	0.413	-0.433	-1.934	0.065
	Cons.			77.065	27.88		2.764	0.012
Survivorship 1 abdomen	DA	UV	H	-0.269	0.1	-0.545	-2.692	0.014
	LA	VIS	C	21.04	7.96	0.535	2.643	0.016
	Cons.			-118.447	89.694		-1.321	0.216
	DC	UVB	B	-0.782	0.283	-0.848	-2.763	0.02
	DC	UVB	H	0.5	0.262	0.439	1.907	0.086
	DC	UVA-Blue	C	-47.801	6.164	-3.019	-7.755	<0.001
	DC	VIS	H	-0.073	0.025	-0.55	-2.958	0.014
	DC	VIS	C	29.733	4.366	2.373	6.81	<0.001
Survivorship 1 carapace	LC	UVB	C	-26.81	4.899	-1.434	-5.472	<0.001
	LC	UVA-Blue	B	-1.28	0.245	-2.139	-5.217	<0.001
	LC	UVA-Blue	C	45.891	6.079	1.645	7.549	<0.001
	LC	VIS	B	0.322	0.059	1.178	5.493	<0.001
	LC	VIS	H	0.138	0.053	0.344	2.579	0.027
	LC	VIS	C	-24.523	5.009	-1.118	-4.896	0.001
	Cons.			8.24	3.527		2.336	0.03
Survivorship 2 abdomen	LA	VIS	H	-0.017	0.006	-0.525	-2.586	0.018
	LA	VIS	C	10.698	5.387	0.403	1.986	0.061
	Cons.			1723.43	498.486		3.457	0.007
Survivorship 2 carapace	DC	UVB	B	-0.915	0.173	-1.469	-5.295	<0.001
	DC	UVB	H	0.789	0.165	1.026	4.789	0.001
	DC	UVA-Blue	C	-35.645	3.785	-3.333	-9.417	<0.001

DC	VIS	H	-0.054	0.022	-0.606	-2.503	0.034
DC	VIS	C	18.309	2.393	2.164	7.65	<0.001
LC	UVB	H	1.354	0.25	1.774	5.426	<0.001
LC	UVB	C	-5.775	1.552	-0.931	-3.722	0.005
LC	UVB	B	-26.274	3.176	-2.081	-8.274	<0.001
LC	UVA-Blue	B	-1.863	0.266	-4.607	-7.006	<0.001
LC	UVA-Blue	C	31.346	4.815	1.663	6.51	<0.001
LC	VIS	B	0.354	0.058	1.914	6.063	<0.001
LC	VIS	C	-18.399	4.029	-1.242	-4.567	0.001

4.3.3.4 Egg number and hatching rates

There was a significant negative correlation between VIS chroma and the number of eggs of the first egg sac as well as between VIS hue and the number of eggs of the first egg sac (**Table 4.8**). Abdomen and dorsal carapace VIS chroma had significantly negative effects on the number of eggs of the first egg sac. Similarly, lateral carapace and abdomen VIS hue had significantly negative effects on the number of eggs of the first egg sac. UVB had a significant positive effects on the number of eggs of the first egg sac. Females that had mated the males with longer DC UVB hue, LC UVB hue and higher LC UVB chroma produced significantly more eggs within the first egg sac **(Table 4.8)**.

Both SD and SM colouration could affect the hatching rate of the first egg sac (R = 0.839, $F_{13, 24}$ = 4.397, P < 0.001) (**Table 4.8**). The eggs produced by the females that had mated the male with bright, longer hue and higher chroma lateral abdomen, higher VIS chroma dorsal abdomen as well as bright UVB lateral carapace were less likely to hatch. For the sexual monochromatic dorsal carapace, the eggs produced by the females that had mated the males with brighter UVB dorsal carapace, longer UVA-Blue hue and higher UVA-Blue chroma were more likely to hatch, but the eggs produced by the females that had mated the males with brighter VIS dorsal carapace were less likely to hatch **(Table 4.8)**. As egg number of the first egg sac largely contributed to the total egg number, the effects of male colouration on the total number of eggs were similar to the effects of the first egg sac as described above (details as **Table 4.8).** The same effects occurred on the total number of spiderlings as well (details as **Table 4.8)**. Total hatching rate can not be predicted by the liner regression model (R = 0.719, $F_{13, 24}$ = 1.974, P = 0.072).

4.3.3.5 Juvenile survivorship

The male colouration in sexual monochromatic dorsal carapace, UVB total UVB brightness, UVA-Blue chroma, VIS hue and chroma had significant effects on juvenile survivorship. The male colouration in sexual dichromatic lateral carapace, UVB chroma, UVA-Blue total brightness and chroma, VIS total brightness and chroma significantly affected juvenile survivorship **(Table 4.8)**. Chromas of UVA-Blue and VIS for both body regions had significant effects on juvenile survivorship. Juveniles of the females that had mated the males with higher dorsal carapace VIS chroma survived better, but juveniles of the females that had mated the males with higher lateral carapace VIS chroma had a poor survivorship. However, juveniles of the females that had mated the males with lower UVA-Blue chroma dorsal carapace survived better but juveniles of the females that had mated the males with higher UVA-Blue chroma lateral carapace survived better **(Table 4.8)**.

4.4 Discussion

Despite being sexually dichromatic in the UV, human-visible (VIS) colours or both on three out of four body regions (DA, DC, LA and LC) and thus likely candidates for honest mating signals, I found that these sexually dichromatic colours of male *C. acerosa* were not good predictors of successful mating. However, the sexually monochromatic dorsal carapace, which did not illustrate any significant difference in all of the three wavelength ranges (UVB, UVA-Blue and VIS) between males and females, was found to be a best predictor of successful mating. Our further analysis of colours, however, illustrated that the successfully mated males had dorsal carapace that showed significantly higher VIS chroma than the males that had failed to mate, but their dorsal carapace colours well matched female dorsal carapace colours. These results suggest that the sexually monomorphic rather than dimorphic colouration plays an indispensable role in determine mating success in *C. acerosa*. These results were unexpected, as favouring a partner with dissimilarity may reduce offspring homozygosity and inbreeding risk (Bernatchez and Landry, 2003; Jennions, 1997; Kamiya et al., 2014; Zeh and Zeh, 1996, 1997). This is, to my knowledge, the first evidence of female mating preference for colour-similar mate in jumping spiders. My results further suggest that in *C. acerosa*, females may obtain bith direct and indirect genetic benefits from sexually monomorphic dorsal carapace VIS chroma based mating strategy. However, dorsal carapace VIS chroma rarely affected mating success alone. In other words, it usually worked with other sexual dimorphic or monomorphic colours together. Interestingly, although mating success is not based on the sexual dimorphic colour of dorsal abdomen, lateral abdomen and lateral carapace, the colours of these body regions are also predictors of male reproduction success.

My finding seems to be inconsistent with overwhelmingly majority of the previous studies which claim that the sexual dimorphic colours are indispensable in sexual selection. For example, prior studies in jumping spiders (Li et al., 2008b; Lim and Li, 2004, 2006a, b; Lim et al., 2007b; Taylor et al., 2011; Taylor and McGraw, 2013b) have showed sexual dimorphic colours are crucial in inter- and intrasex interaction. There are several possible interpretations for this discrepancy. First, the coexistence of sexually dichromatism and monochromatism may be common in many jumping spider. However, the majority of previous studies have primarily focused on male-biased ornamental colours and particularly on sexually dimorphic colours in salticids such as the ornament red face in male of *Habronattus pyrrithrix* (Taylor and McGraw, 2013a) and UVB in male of *Phintella vittata* (Li et al., 2008b). However, *C. acerosa* in my investigation is exhibiting ornament colours not only in males but also in females. Therefore, I assume that mutual mate-choice may be the driving force of this mutual ornamentation (i.e., dorsal carapace) in *C. acerosa,* while female mate-choice may have driven the male-biased, sexually dichromatic ornamentation as mentioned above. Another explanation for female mating preference for colour-similar mate is that the colour contrast (Burns and Dalen, 2002) was lost when the sexually dimorphic UV was blocked from males of *P. vittata* or *Cosmophasis umbratica* (Li et al., 2008b; Lim and Li, 2006a; Lim et al., 2008b), hence the correspondence females could not recognise the UV-blocking males as their conspecific males, and paid less attention to males with UV removed but more attention to the males without UV removed. I am making this suppose because although the UV colouration did not improve successful mating in *C. acerosa*, the females are found to pay less attention to the males when their UV colour was blocked (Chapter 3).

The mechanism and adaptation of this monochromatic ornamental colouration based mating preference are controversial. According to the genetic correlation hypothesis, the elaborate monomorphic traits are functional only in males, but they are merely non-functional by-products for females as a result of between-sexes genetic correlations (Lande, 1987; Rice, 1984). While mutual selection hypothesis states that the sexually monomorphic traits can arise from selection for their expression in both males and females (Johnstone, 1997; Johnstone et al., 1996; West-Eberhard, 1979). However, one recent study by Kraaijeveld et al. (2007) indicated that sexually mutual ornaments can have a signal function in both sexes, especially during mate choice. Previous studies illustrate that both males and females probably make their mating decisions based on the ornament mutual sexual ornamentations (Berglund et al., 2005). However, this mutual ornament based mating preference can be changed according to environmental conditions like seasonal (Chaine and Lyon, 2008) or temperature (Prudic et al., 2011) differences. Similar evidence also has been found in insects (Gwynne and Simmons, 1990). For example, a study on bush crickets provides evidence that courtship-role can be reversal dependent on the availability of food resource (Simmons and Gwynne, 1993). All of these provided the possibility that mutual sexual shared ornamentations can be driven or maintained through mutual mating choice. In addition to behavioural mutual mate-choice, mutual sexual ornamentations still can be driven by genetic correlation (Lande, 1980), intrasex competition (Gwynne and Simmons, 1990) and developmental plasticity (Prudic et al., 2011).

Selection for colour-similar males can be adaptive. Recent studies showed that more major histocompatibility complex (MHC) similar males sire more offspring in guppies (*Peocilia reticulata*) under competitive conditions (Fitzpatrick et al., 2014;

Gasparini and Lai, 2015). Other studies have also suggested that in fish females could benefit from avoiding males too different from themselves to produce offspring with an optimal intermediate allelic diversity which may confer a better pathogen resistance (Innes et al., 2008; Reusch et al., 2001). Moreover, selection for MHC-similar males in may be adaptive in the light of avoiding hybridization as suggested in a study of Atlantic salmon (Yeates et al., 2009). If females select against males of different populations of females, preference of VIS-chroma similarity could be the underlying mechanism. However, why females are more likely to mate with males with mutual monomorphic colours C. acerosa remains untested. Furthermore, as I only examined the correlation between male ornamental colouration and male mating success without giving females a choice, more evidence is still need from experiments involving female mate choice.

The mating-based dorsal carapace VIS chroma of males is shown to be a god predictor of courtship latency, the number of eggs, and juvenile survivorship in *C. acerosa*. Male DC-VIS chroma is positively linked to courtship latency and juvenile survivorship, but there is a negative association between this chroma and the number of eggs. Therefore, these results illustrate that DC-VIS chroma based mating strategy is selected by this species most probably as a result of achieving both direct and indirect benefits. Previous studies have shown that elaborate male colouration usually acts as a signal of its quality in birds (Hill, 1990, 1991a; Sundberg and Larsson, 1994) and fish (Houde, 1987; Nicoletto, 1995), and this quality may supply genetic benefits in terms of increase juvenile quality (Lande, 1987). In socially monogamous species, male may supply benefits by parental care, however, in species such as *C. acerosa*, it is unlikely to happen as males do not provide any parental care. Hence most probably, the sexually monochromatic DC-VIS chroma based mating strategy is closely

connected to genetic benefits provided by males or both sexes. However, DC-VIS chroma is negatively associated to the number of eggs. This probably can be explained that fewer number of eggs may reflect higher egg quality, which is supported by a recent study in a bird species (Robinson et al., 2014).

As mentioned, sexual dichromatic and sexual monomorphic colours usually work together to affect the reproduction success of *C. acerosa*. However, the results showed that SM colours play more important role in contributing to egg production, increasing egg hatching, number of spiderlings and juvenile survivorship. However, in terms of increasing number of egg-sacs, courtship latency, copulation duration and egg hatching rate, both SM and SD colours usually play a similar role. It can probably be interpreted as different ornamentations may advertise different aspects of male qualities, which has been supported by studies in birds (Johnstone, 1995; Robinson et al., 2014), and the final mating decision usually is made based on multiple signals overall evaluation (Candolin, 2003; Iwasa and Pomiankowski, 1994) or different indicator will be selected by mating choice based on different conditions (Candolin, 2003; Taylor and McGraw, 2013b). For example, Taylor and McGraw (2013b) have shown that in a jumping spider species (*Habronattus pyrrithrix*), the ornament red colouration on male face can just improve mating success when the sunlight is supplied. Therefore, multiple ornamentations probably can make sure the availability of alternative indicators for mating decision in *C. acerosa*. I also found positive effects usually integrate with negative effects together to affect one reproduction aspect. For example, the UV total UV brightness of lateral abdomen was positively associated with copulation duration. However, there was a negative relationship between VIS brightness and copulation duration for the same body region. Perhaps ornaments may advertise either positive or negative effects in reproduction success,

and the reproduction success is based on overall effects rather than a single effect. This has been supported by a previous study in birds (Amundsen, 2000; Bonato et al., 2009; Iwasa and Pomiankowski, 1994; Johnstone, 1995). If this hypothesis is true, the reproductive success is not only dependent on discern the positive contribution ornamentations but also relying on the distinction and avoidance of negative contribution ornaments.

Although the results have shown that females can get indirect benefits through select males based on their dorsal carapace VIS chroma in *C. acerosa*, it is unclear whether this chroma is heritable or not and where this chroma will play a function in improve mating success for the next generation. Hence, it is necessary to conduct further studies in the offspring generation to answer these questions.

In conclusion, my results illustrate that the monomorphic ornamental colouration of dorsal carapace improves successful mating of males in the salticid spider *C. acerosa* that has multiple body regions exhibiting elaborated sexually dimorphic colours. This preference for more colour-similar males is in contradiction with the expected advantage as assumed by the ‘genetic compatibility hypothesis. This study suggests that the preference for colour-similar mates may be common in salticids and in invertebrates.

CHAPTER 5

The evolution of sexual dimorphic colouration in jumping spiders of the subfamily Heliophaninae (Araneae: Salticidae)

5.1 Introduction

Sexual dimorphism is a phenotypic difference between male and female of the same species. It is a widespread phenomenon which spans across the different groups of sexual organisms and it attracts a great interest to both evolutionary biologists and behavioural biologists. Since 1990, there has been a surge in the number and frequency of papers relating to the topic of sexual selection and speciation (Arnqvist, 1998; Arnqvist et al., 2000a; Gage et al., 2002b; Kirkpatrick and Ravigné, 2002; Maan and Seehausen, 2011; Manier et al., 2013; Ritchie, 2007c; Rodríguez-Muñoz et al., 2010; Singh and Singh, 2013; van Doorn et al., 2009). Sexual selection can occur before (pre-copulation), during (peri-copulation) and after copulation (post-copulation) (Andersson, 1994). It seems highly likely that sexual selection contributes to the divergence of the characters which is directly connected to sexual isolation in allopatry (Ritchie, 2007a). In other words, speciation is possibly a result of both intra- and intersexual competition which can occur at all three phases of sexual selection (Andersson, 1994). Furthermore, sexually selected characters evolve rapidly and often vary at a large scale among closely related species, and comparative studies have showed that sexually selected taxa exhibit a high level of species diversity (Kraaijeveld et al., 2011). Rowe and Arnqvist (2012) claim that sexual selection is the major cause for speciation events, and it is one of the most critical forces driving reproductive isolation which further results in the origin of new species. In several fish species, behavioural variations arised more rapidly than the variations in genital structure, suggesting that these species have very complex courtship behaviour (Mendelson, 2003). However, according to (Ritchie, 2007a), whether sexual selection should be responsible for most of the speciation events remains controversial. Some biologists claim that sexual selection is likely to be responsible for the origin of new

species (Arnqvist et al., 2000b; Eberhard, 1985, 1990), and they believe that the most closely related species can merely be distinguished by the sexually dimorphic traits. In some arthropods, characters related directly to sexual functions evolve much faster than other indirectly related characters (Clark and Dudley, 2009; Ritchie, 2007c). However, these studies are derived mainly from metadata analyses, in which the data have been collected by taxonomists but not by themselves. The original purposes of these data were for species identification and distinction only, which is often done within subsets of the various taxonomic groups in isolation. Furthermore, the resolution of the characters studied is also likely to be inadequate for evolutionary studies involving higher taxonomic groups. As such, dedicated studies are necessary for more reliable and meaningfully comparable data as proposed here (Ritchie, 2007a).

Jumping spiders (Araneae: Salticidae) are ideal model system for several reasons. First, jumping spiders have a high level of species diversity with 5814 species from 587 genera (World Spider Catalog, 2015). Second, a majority of the species show sexually dimorphic colours. Third, these spiders have highly acute vision (Land and Barth, 1992) and can also perceive UV spectrum in addition to human visible colours (De Voe, 1975a; Peaslee and Wilson, 1989), which enables colouration as a clue for both inter- and intrasex communication. Furthermore, most of the species have very complex courtship behaviour (Oxford and Gillespie, 1998b; Richman and Jackson., 1992). Taken together, these make jumping spiders ideal taxa for the study of colour evolution (Oxford and Gillespie, 1998a). Colour manipulation in male salticids can strongly affect female mating preference (Peckham and Peckham, 1894). Lim and Li (2006b) indicated that there is a strong sexual dimorphism in UV reflectance in the salticid *Cosmophasis umbratica*. Another species of salticid, *Phintella vittata*, has also been found to have extreme sexual dimorphism in UV

reflection, and this character plays a key role in female mate choice (Li et al., 2008b). These imply that in salticids, colouration (including the UV range) is likely selected by females (or males) in choosing mates. However, there is no study investigating the evolution of sexual dichromatism in these taxa, thus we proposed such a study across multiple of species in the target subfamily of salticide spiders. Studying the evolution of colour patterns requires mapping colour onto the phylogenetic hypotheses (Ingram and Parker, 2008). The UV and other human-visible (VIS) colour measurements are continuous characters that can be mapped onto phylogenetic trees using a variety of different techniques (Goloboff and Giannini, 2008; Goloboff et al., 2006; Maddison and Maddison, 1992).

In this study, I first reconstructed molecular phylogeny of the subfamily Heliophaninae, and then quantified sexual dichromatism and colours of multiple species in this subfamily. Finally, by mapping spectral reflectance components (total brightness, hue and chroma) of UV and VIS to the phylogenetic tree, I attempted to trace the evolutionary history of colouration within this clade of salticids.

5.2 Materials and methods

5.2.1 Taxa sampling and specimen preservation

The dataset comprised 40 species, including 38 species from nine genera of the subfamily Heliophaninae (Maddison and Hedin, 2003), two outgroup species from subfamily Plexippinae (**Table 5.1** and **Fig. 5.3**). My sample targeted especially the genus *Phintella* from East and South-east Asia, in which 19 species were included in this study.

I fixed adults in absolute ethanol, then stored their legs at -80 ℃ for subsequent DNA extraction, and preserved the remaining of the voucher in 80% ethanol for identification. I used small juveniles entirely for DNA extraction. I brought large juveniles and subadults to the laboratory and reared them individually till maturation in cages (diameter × height: 50 × 60 mm). The laboratory rearing environment was set to resemble the environmental conditions (temperature: 25 ± 1 ℃; relative humidity: 80 ± 5%; light cycle: 12h /12 h from 0800-2000 h). Upon maturation, I fixed the reared specimens as above after we had finished all the colour measurements (Chapters 2, 3 and 4) and behavioural experiments (Chapters, 3, 4 and 5). All vouchers are deposited at LKC Natural History Museum, National University of Singapore.

I identified adults based on genital morphology from the primary literature and Monograph of Salticidae (Araneae) of the World (Prószyński, 2014). I sampled and identified 23 known heliophanine species and 17 unknown heliophanine species (4 *Cosmophasis*, 1 *Heliophanus*, 1 *Icicus*, 8 *Phintella*, and 3 *Siler*) **(Table 5.1).**

Table 5.1. Species that have been used for genetic phylogeny reconstruction in this study and the primers that have been used for PCR (y = Yes, n = No).

Specimen code	Genus	Species	Country	Locality	Lat/Long	COI	28S	Actin 5C	16S/ND1
P-B-M5	*Phintella*	*bifurcilinea*	China	Hunan, Xiangxiang	27.532/109.060	y	y	y	n
P-B-S1	*Phintella*	*bifurcilinea*	China	Guangdong, Guangzhou	23.108/113.178	y	y	y	y
P-B-M14	*Phintella*	*bifurcilinea*	China	Hainan, Exianling	19.019/109.060	y	y	y	y
P-B-18	*Phintella*	*bifurcilinea*	China	Yunnan, Liuku	25.520/98.513	y	n	y	y
P-B-M21	*Phintella*	*bifurcilinea*	China	Yunnan, Gongshan	27.448/98.403	n	n	y	y
P-B-M12	*Phintella*	*bifurcilinea*	China	Hainan, Jianfengling	18.443/108.520	y	n	y	y
P-B-XTBG	*Phintella*	*bifurcilinea*	China	Yunnan, Xishuangbanna	21.557/101.154	y	y	y	y
P-B-F-Nantou	*Phintella*	*bifurcilinea*	China	Taiwan, Nantou	23.581/120.534	y	y	n	y
P-F01-01	*Phintella*	sp. 1	China	Yunnan, Gongshan	28.013/98.378	y	n	n	n
P-F4-01	*Phintella*	sp. 3	China	Hubei, Huangpi	31.058/114.278	y	y	y	y
M21-P-J-1	*Phintella*	*levii*	China	Yunnan, Gongshan	27.448/98.403	y	y	y	n
M23-P-I-F	*Phintella*	sp. 2	China	Yunnan, Gongshan	28.012/98.368	y	y	y	y
P-G-F-08	*Phintella*	*linea*	China	Hubei, Tongshan	29.246/114.399	y	y	y	n
P-E-F-01	*Phintella*	*cavaleriei*	China	Henan, Xixia	33.317/111.405	y	n	y	n
P-F-F-01	*Phintella*	*arenicolor*	China	Hunan, Changsha	27.585/23.005	y	y	y	n
P-A-F-07	*Phintella*	sp. 4	China	Yunnan, Xishuangbanna	21.556/101.161	y	y	y	y
P-L-F-01	*Phintella*	*assamica*	Thailand	Wang Yao, Vimandin	14.118/099.154	y	y	n	n
P-K-F-02	*Phintella*	sp. 5	Thailand	Nong Bua	12.533/101.160	y	y	n	y
P-K-M-01	*Phintella*	*clathrata*	Thailand	Wang Yao, Vimandin	14.118/099.154	n	y	y	n
P-M-M-01	*Phintella*	*argenteola*	Singapore	Botanical garden	1.184/103.486	y	y	n	y
P-Nantou-M	*Phintella*	sp.9	China	Taiwan, Nantou	23.595/120.513	y	y	y	y
P-F03-02	*Phintella*	*abnormis*	China	Hunan, Longtan	27.532/112.305	y	n	n	n
P-F02-01	*Phintella*	sp.12	China	Yunnan, Gongshan	27.591/98.334	y	y	n	n

Ch-V-S10	*Phintella*	*versicolor*	Singapore	Labrador Nature Reserve	1.172/103.485	y	y	n	n
Ch-V-Xianghua	*Phintella*	*versicolor*	China	Hunan, Xiangtan	27.532/112.305	y	n	n	n
M15-07	*Phintella*	*versicolor*	China	Hainan, Exianling	19.019/109.060	y	n	n	n
Ch-V-S6	*Phintella*	*versicolor*	China	Hubei, Wuhan	19.020/109.059	y	n	y	n
M16-13	*Phintella*	*versicolor*	China	Hainan, Bawangling	19.052/109.073	y	n	n	n
Ch-V-M13	*Phintella*	*versicolor*	China	Hainan, Jianfengling	18.421/108.474	y	n	n	n
Ch-v-M11	*Phintella*	*versicolor*	China	Guangdong, Heyuan	23.450/114.434	y	n	n	n
Ch-V-S4	*Phintella*	*versicolor*	China	Guangdong, Guangzhou	22.597/113.234	y	n	n	n
Ch-V-M25	*Phintella*	*versicolor*	China	Yunnan, Xishuangbanna	21.556/101.161	y	n	y	y
P-V-M-3	*Phintella*	*vittata*	China	Guangdong, Shenzhen	22.349/114.105	n	y	y	y
P-V-M12	*Phintella*	*vittata*	China	Hainan, Jianfengling	18.421/108.474	n	n	n	y
P-V-M25	*Phintella*	*vittata*	China	Yunnan, Xishuangbanna	21.556/101.161	y	y	y	y
P-V-Singapore	*Phintella*	*vittata*	Singapore	Pulau Ubin Island	1.244/103.571	n	y	y	n
P-V-M-KL	*Phintella*	*vittata*	Malaysia	Changlun Kedah, UUM	6.275/100.301	n	y	n	y
Chrycilla-Cq	*Chrysilla*	*acerosa*	China	Chongqing, Jiyunshan	29.502/106.239	y	y	y	n
Chrysilla lauta	*Chrysilla*	*lauta*	Thailand	Nakhon Ratchasima	14.515/102.053	y	y	y	n
Epocilla	*Epocilla*	*calcarata*	China	Jiangxi, Ganzhou	28.549/114.538	y	n	y	n
Ep-sp.2-M-01	*Telamonia*	*climidiata*	Malaysia	Selangor, Kemensah	3.131/101.471	y	y	y	n
Ep-sp.2-F-02	*Telamonia*	sp.1	Malaysia	Selangor, Kemensah	3.131/101.471	y	y	y	n
Ep-sp.1-F-01	*Epocilla*	*calcarata*	Malaysia	Changlun Kedah, UUM	6.275/100.301	y	y	y	y
Heliophanus pair	*Heliophanus*	sp.	Singapore	Dover Vista Park	1.176/103.465	y	y	y	y
H-01-F	*Plexippine*	sp.	China	Hainan, Jianfengling	18.421/108.474	y	y	y	y
Icicus pair	*Icicus*	sp.	Singapore	Dover Vista Park	1.176/103.465	y	y	y	y
Orsima ichneumon	*Orsima*	*ichneumon*	Malaysia	Selangor, Gunuang Nuang	3.156/101.536	n	n	y	y
Menererus	*Menemerus*	*bivittatus*	Singapore	NUS	1.175/103.463	y	y	y	n
C1-F-03	*Cosmophasis*	*umbratica*	Singapore	Labrador Nature Reserve	1.172/103.485	y	n	y	y
C1-M-Thai-01	*Cosmophasis*	*umbratica*	Thailand	Nong Ki	14.101/101.501	n	n	y	y

C2-F-02	*Cosmophasis*	sp. 1	Singapore	Labrador Nature Reserve	1.172/103.485	y	y	y	y
C3-F-01	*Cosmophasis*	*lami*	Singapore	Labrador Nature Reserve	1.172/103.485	y	y	y	y
C3-F-02	*Cosmophasis*	sp.4	Malaysia	Kuala Terengganu	5.181/103.063	y	n	n	y
C6-F-01	*Cosmophasis*	*lami*	Singapore	Labrador Nature Reserve	1.172/103.485	y	n	y	n
C8-Pair-China	*Cosmophasis*	sp. 3	China	Hainan, Exianling	19.019/109.060	n	n	n	y
S1-F-01	*Siler*	*semiglaucus*	China	Hainan, Jianfengling	18.443/108.520	y	n	n	n
S3-F-01	*Siler*	sp. 1	China	Hainan, Bawangling	19.052/109.073	y	y	y	n
S4-F-01	*Siler*	*collingwoodi*	China	Yunnan, Xishuangbanna	21.556/101.153	y	y	y	n
S5-F-01	*Siler*	sp. 2	Singapore	Labrador Nature Reserve	1.172/103.485	y	y	y	n
S6-F-01	*Siler*	sp3	China	Xiamen, Yundingshan		y	n	y	y
S8-F-01	*Siler*	*cupreus*	China	Fujian, Putian, Xianyou		n	y	y	y
S9-F-01	*Siler*	*semiglaucus*	China	Fujian, Putian, Xianyou		n	y	n	y

5.2.2 Molecular procedures

I extracted DNA from spider legs using a modified CTAB extraction protocol (Shahjahan et al., 1995). I followed the standard spider phylogenetic choice of loci and standard polymerase chain reaction (PCR) settings (Maddison and Hedin, 2003; Maddison et al., 2014; Zhang and Maddison, 2013). Five genes were amplified for this analysis. One nuclear ribosomal gene, 28S (Hedin and Maddison, 2001), one nuclear protein coding gene Actin 5C, and two mitochondrial regions cytochrome c oxidase subunit 1 (CO1) and another region that includes 16S and NADH dehydrogenase I (ND1) ("16S/ND1", Hedin and Maddison 2001).

PCR was used to amplify DNA fragments from four loci (primers in **Table 5.2**). Cycling conditions for the primers started with an initial 95˚C denaturation, followed by 30 cycles of 1 minute at 95˚C, 1 minute at 55˚C (28S, Act 5C), 46˚C (16S/ND1) or 48˚C (CO1) and 1.5 minutes at 72˚C, and a final 2 minutes extension at 72˚C. PCR was carried out using the Hotstart Ex-Taq (Takara, Japan). The amplified fragment was purified with SureClean (Bioline, USA). The purified PCR products were sequenced directly in both directions using an ABI 3100 (Applied Biosystems).

Chromatograms were combined and edited using Sequencher 4.6 (Gene Codes Corp.). All genes were aligned using MAFFT v7 on an Intel Xeon CPU E5-2630. Sequences were checked for likely contamination using SequenceMatrix 1.7.8 to identify interspecific distances of < 0.5% for fast evolving genes (Su et al 2008). Protein coding genes were translated to amino acids and checked for stop codons using MEGA 5.2. Gene partitions were concatenated using SequenceMatrix (Su K. F.Y et al., 2008). The final matrix was analysed as four partitions (i.e. 28S, Act 5C, COI and 16S/ND1).

Table 5.2. List of primers used with annealing temperatures.

Gene	Primer	Reference
CO1	CI-J-2309	(Masta, 2000)
	CI-N-2776	(Maddison and Hedin, 2003)
16S/ND1	NI-J-12261	(Maddison and Hedin, 2003)
	LR-N-12945	(Maddison and Hedin, 2003)
28S	28S-O	(Maddison and Hedin, 2003)
	28S-C	(Maddison and Hedin, 2003)
	Act-MBF2	
Act 5C	Actin-R-1009	(Bodner and Maddison 2012)

5.2.3 Phylogenetic analyses

I used model based alogorithns to find optical trees from the full matrix. Selection of best-fit models of nucleotide substitution for each partition was analysed using JModelTest v 2.1.7 based on the Akaike Information Criteria (AIC) (Posada, 2008). Likelihood calculations were carried out for 88 candidate models, which included 11 substitution schemes, including models with equal or unequal base frequencies, a proportion of invariable sites and rate variation among sites. If a model selected under the AIC could not be implemented in MrBayes, the least complex model that included all of the parameters of the selected model and could be implemented, was used instead. I performed maximum likelihood (ML) analyses in Garli v2.01 (Zwickl, 2006). The best-fit models were used for maximum likelihood (ML) as COI: TVM+I+G, 16S/ND1: GTR+I+G, 28S: GTR+I+G, Act5C: TVM+G. ML analysis ran for 50,000 generations with 10 searchreps for getting the best tree, and assessed branch supports by 500 bootstrap replicates with 2 searchreps. I used SumTrees in the DendroPy phylogenetic Python library (Sukumaran and Holder, 2010) for generating a majority-rule bootstrap consensus tree. I conducted Baysian inference (BI) in MrBayes v3.2.5 (Ronquist et al., 2012) by running Markov chain Monte Carlo

(MCMC) for 43,000,000 generations, and 2 independent runs reached stationarity. The models used for BI in MrBayes were COI: GTR+I+G, 16S/ND1: GTR+I+G, 28S: GTR+I+G, Act5C: GTR+G. Details of methods in the genetic phylogeny reconstruction were following the previous studies (Zhao et al., 2013).

5.2.4 Reconstruction of colour evolution

I performed Mesquite (Maddison and Maddison, 2012) for data manipulation and to trace character evolution. For ancestral-state reconstruction, the parsimony criterion was used as implemented in Mesquite, as any model-based methods would 'evolve traits all over the tree for biologically less-meaningful interpretations. The data on sexual dichromatism in the UV and VIS range of heliophanine salticids used here were obtained from **Chapter 2** (*Phintella* species) and **Chapter 3** (*Chrysilla acerosa*) as well as from previous studies of *Cosmophasis umbritica* (Lim and Li, 2006b). The dataset provided the following characters that were mapped onto the phylogenetic tree using Mesquite (**Tables 5.3 - 5.6**): (1) Sexual dichromatism in the UV wavelength range (300-400 nm): absent = 0; present = 1 (Chapters 2 and 3, Lim and Li 2006a). (2) Sexual dichromatism in the VIS Wavelength range (400-700 nm): absent = 0; present = 1 (Chapters 2 and 3). In the case of species with no available specimens for colour measurements, the data sources are marked as unknown. The evolution of male colouration (total brightness: Qt; hue and chroma) of four main body regions (dorsal abdomen, dorsal carapace, lateral abdomen and lateral carapace) (**Figs. 5.1** and **5.2; Tables 5.3 and 5.5)** was reconstructed as continuous variable, and for each body region separately. If there is present sexual dichromatism, I marked it as "1", and if no sexual dichromatism, I marked it as "0", then the matrix has been input to software of Mesquite 2.75 (Maddison and Maddison, 2012) and mapped it to restored (but modified) phylogeny.

In the second stage, I focused on two body regions (dorsal abdomen and dorsal carapace) of male to analyse the evolutionary history of UV and VIS by mapping Qt (total brightness), hue and chroma separately to the phylogenetic tree.

Table 5.3. Spectral reflectance in the body region of dorsal abdomen in heliophaninae species

Species	Sex	N	Dorsal abdomen					
			UV (300-400 nm)			VIS (400-700 nm)		
			Qt	Hue	Chroma	Qt	Hue	Chroma
Cosmophasis lami	Female	2	18.3 ±2	300 ±0	0.335 ±0.035	153.6 ±5.2	698.5 ±0.5	1.635 ±0.105
Cosmophasis-Thai	Female	1	29.4	300	0.58	156	695	1.51
Cosmophasis umbratica	Female	5	45.5 ±7.7	313.6 ±5	0.386 ±0.202	187.5 ±12.7	628.2 ±28.2	0.998 ±0.211
	Male	2	48.2 ±1	317.5 ±1.5	0.05 ±0.01	183.9 ±1.6	582 ±0	0.885 ±0.025
***Cosmophasis* sp. 1**	Female	2	66.3 ±10.7	302.5 ±1.5	0.235 ±0.015	242 ±4.7	696 ±0	0.53 ±0.14
Chrysilla acrosa	Female	20	64.2 ±0.8	300 ±0	0.465 ±0.03	194.5 ±3.3	471.2 ±2.2	0.472 ±0.025
	Male	20	61.2 ±1	301.1 ±1.1	0.535 ±0.025	167.9 ±5	486.4 ±6.3	0.505 ±0.03
Chrycilla lauta	Female	1	43.5	312	0.3	183.7	699	0.97
	Male	1	33.5	300	0.97	119.6	533	2.37
Epocilla calcarata	Female	1	69	300	0.37	160.8	700	0.58
	Male	1	67.7	310	0.91	140.9	698	1.3
Phintella. arenicolor	Female	5	25 ±2.3	323.4 ±3.6	0.265 ±0.031	240.1 ±2.3	616 ±12.2	0.972 ±0.036
	Male	5	46.1 ±7.1	353 ±16.2	0.155 ±0.018	46.1 ±7.1	353 ±16.2	0.155 ±0.018
P. bifurcilinea	Female	5	53.3 ±3.9	337 ±16.4	0.226 ±0.07	231.5 ±10.3	638.4 ±20.5	0.687 ±0.092
	Male	5	47.1 ±5.6	382.1 ±14.6	0.219 ±0.036	258.5 ±5.8	587.8 ±16.1	0.571 ±0.089
P. cavaleriei	Female	5	33.9 ±5.3	337.4 ±14.7	0.123 ±0.02	234 ±10.2	672 ±23	0.866 ±0.114
	Male	5	29.6 ±1.4	359.4 ±18.3	0.238 ±0.033	235.5 ±3.2	659.4 ±21.5	0.885 ±0.039
P. clathrata	Female	1	93.7	304	0.12	277.5	580	0.11
P. linea	Female	5	47.5 ±4	300.8 ±0.6	0.323 ±0.037	244.5 ±6.3	649.9 ±28.3	0.742 ±0.069
	Male	5	64.8 ±3.8	366.9 ±19.6	0.191 ±0.04	272 ±4.4	625 ±37.7	0.352 ±0.046

P. vittata	Female	5	13.6 ±1.3	303.8 ±3.1	1.083 ±0.138	172.6 ±6.2	613.7 ±12.9	1.601 ±0.068
	Male	5	28.7 ±13.5	314.8 ±14.8	0.65 ±0.122	200.2 ±18.4	602.6 ±22.1	1.209 ±0.246
***Phintella* sp. 4**	Female	5	52.7 ±5.8	325.7 ±18.3	0.161 ±0.04	256.7 ±4	632 ±26.5	0.571 ±0.074
	Male	5	60.7 ±9.3	375 ±16.6	0.243 ±0.045	265.4 ±10.3	551.6 ±44.2	0.432 ±0.135
***Phintella* sp. 6**	Female	5	64.8 ±1.2	303.3 ±0.7	0.157 ±0.008	264.5 ±1.2	580 ±0	0.446 ±0.016
	Male	5	47.7 ±1.8	300.6 ±0.1	0.233 ±0.015	241.7 ±2	694.6 ±0.3	0.705 ±0.03
***Phintella* sp. 8**	Female	5	72.9 ±3.3	395.1 ±0.7	0.234 ±0.006	279.2 ±2.4	529.5 ±16.4	0.228 ±0.019
	Male	5	276.3 ±3.1	509 ±26.5	0.273 ±0.055	244.2 ±9.3	516 ±42.9	0.583 ±0.132
***Phintella* sp. 11**	Female	1	59.8	300	0.47	248.1	696	0.62
***Phintella* sp. 13**	Female	4	50.5 ±2.4	376.2 ±10.9	0.2 ±0.017	230.7 ±5.8	622 ±14.8	0.602 ±0.064
***Phintella* sp. 14**	Female	5	52 ±6.5	300 ±0	0.388 ±0.071	252 ±4	568.9 ±35.1	0.696 ±0.08
	Male	5	79.3 ±6	300 ±0	0.301 ±0.046	242.9 ±6	540.3 ±17	0.366 ±0.09
Orsima ichneumo	Female	1	69	300	0.37	160.8	700	0.58
	Male	1	67.7	310	0.91	140.9	698	1.3
Siler semiglaucus	Female	2	23.7 ±1.4	300 ±0	0.359 ±0.042	149.6 ±4.8	699.5 ±0.5	1.6 ±0.042
	Male	4	47.1 ±7.6	359.7 ±21.8	0.473 ±0.106	184 ±13.3	691 ±8.3	0.762 ±0.149
S. collingwoodi	Female	2	39.6 ±2.4	301 ±1	0.368 ±0.015	194.7 ±16.4	699 ±1	1.043 ±0.088
***Siler* sp. 1**	Female	5	50.2 ±2.7	301.6 ±1.1	0.386 ±0.042	211.6 ±3.6	669.8 ±27.9	0.666 ±0.065
	Male	1	60.9	379	0.464	224.2	527	0.685
***Siler* sp.2**	Female	2	51.8 ±4.5	300 ±0	0.502 ±0.161	231.4 ±2.9	666.5 ±31.5	0.735 ±0.041
***Siler* sp.4**	Female	1	52.3	300	0.308	198.1	699	0.623
Telamonia climidiata	Female	1	90.1	313	0.22	247.8	685	0.29
	Male	1	31.5	309	0.77	220.3	678	1.11

Table 5.4. Spectral reflectance in the body region of lateral abdomen in heliophaninae and out group species.

Species	Sex	N	Lateral abdomen					
			UV (300-400 nm)			VIS (400-700 nm)		
			Qt	Hue	Chroma	Qt	Hue	Chroma
Cosmophasis lami	Female	2	29 ±5	300 ±0	0.205 ±0.035	198.2 ±7	697.5 ±1.5	1.085 ±0.115
Cosmophasis thai	Female	1	77.6	300	0.4	225	695	0.23
Cosmophasis umbratica	Female	5	52.7 ±3.52	306.4 ±2.6	0.236 ±0.106	201.8 ±8.5	626.6 ±27.3	0.804 ±0.091
	Male	2	81.5 ±0.95	310.5 ±0.5	0.04 ±0	254.7 ±1.8	582 ±0	0.245 ±0.015
***Cosmophasis* sp. 1**	Female	2	64 ±18.8	300 ±0	0.15 ±0.06	246.7 ±22.1	694.5 ±0.5	0.515 ±0.235
Chrysilla acrosa	Female	20	68.1 ±2.39	300 ±0	0.62 ±0.018	235.7 ±3.1	620.2 ±0	0.606 ±0.039
	Male	20	75 ±1.75	300 ±0	0.676 ±0.023	196.6 ±7.6	550.9 ±17.2	0.367 ±0.023
Chrycilla lauta	Female	1	37.8	300	0.29	192.4	695	0.94
	Male	1	39.6	337	0.87	125.8	513	2.08
Epocilla calcarata	Female	1	58.4	382	0.31	206.2	633	0.92
	Male	1	61.6	309	1.15	112.6	700	1.52
P. arenicolor	Female	5	35.3 ±3.82	347.6 ±18.1	0.264 ±0.086	248.9 ±7	624.7 ±22	0.773 ±0.079
	Male	5	45.2 ±1.35	353.2 ±16	0.191 ±0.05	263.2 ±1.2	629.1 ±20.1	0.609 ±0.02
P. bifurcilinea	Female	5	66.7 ±8.18	339.4 ±18.5	0.162 ±0.05	257.9 ±9.5	629.2 ±37.9	0.439 ±0.102
	Male	5	65 ±3.67	395.9 ±1	0.226 ±0.027	273.8 ±3.3	530.6 ±6.9	0.3 ±0.046
P. cavaleriei	Female	5	46.5 ±5.32	397 ±0.5	0.308 ±0.056	260.8 ±5.3	612.8 ±18	0.549 ±0.06
	Male	5	37.2 ±2.46	357.8 ±17	0.303 ±0.076	244.2 ±7.1	637.6 ±25.9	0.737 ±0.08
P. clathrata	Female	1	85.7	300	0.26	244.2	453	0.15
P. linea	Female	5	47.1 ±5.07	325.8 ±16.2	0.217 ±0.036	249.4 ±4.5	623.3 ±26.3	0.677 ±0.092
	Male	5	55.5 ±4.58	359.1 ±15.9	0.182 ±0.057	259.7 ±6	666.3 ±30.4	0.494 ±0.061
P. vittata	Female	5	26.2 ±4.28	338.2 ±22.9	0.634 ±0.093	204.6 ±12.1	610.6 ±13.3	1.12 ±0.156

	Male	5	17.8 ±2.74	304 ±4	0.807 ±0.235	189.4 ±2.7	567 ±6.9	1.341 ±0.05
	Female	5	72.5 ±6.77	361.8 ±17.8	0.117 ±0.018	275.2 ±2.4	638 ±34.4	0.306 ±0.063
***Phintella* sp. 4**	Male	5	79.5 ±4.72	385.6 ±5.2	0.318 ±0.1	237.3 ±11.9	515 ±52.3	0.45 ±0.098
	Female	5	51.2 ±1.96	300 ±0	0.383 ±0.031	246.6 ±2.1	685.1 ±0.9	0.693 ±0.033
***Phintella* sp. 6**	Male	5	44.6 ±4.73	300 ±0	0.207 ±0.047	243 ±4.8	688.6 ±2.2	0.655 ±0.07
	Female	5	76.9 ±4.65	395.5 ±1	0.275 ±0.022	275.3 ±1	488.8 ±23.7	0.244 ±0.014
***Phintella* sp. 8**	Male	5	84.9 ±4.66	347.2 ±20.6	0.069 ±0.009	264.2 ±4.1	567.6 ±60.7	0.314 ±0.032
***Phintella* sp. 11**	Female	1	81.3	300	0.35	234.9	471	0.23
***Phintella* sp. 13**	Female	4	42.5 ±5.21	397 ±0.7	0.275 ±0.045	238.1 ±9.4	608.5 ±11.5	0.655 ±0.059
	Female	5	70.1 ±7.07	300 ±0	0.261 ±0.053	263.8 ±8.2	579 ±34.9	0.442 ±0.107
***Phintella* sp. 14**	Male	5	77.3 ±1.27	300 ±0	0.538 ±0.026	198.1 ±26.1	502.2 ±41.7	0.471 ±0.057
	Female	1	58.4	382	0.31	206.2	633	0.92
Orsima ichneumo	Male	1	61.6	309	1.15	112.6	700	1.52
	Female	2	59.1 ±8.81	350 ±50	0.415 ±0.134	216.1 ±3.1	562.5 ±135.5	0.697 ±0.121
Siler semiglaucus	Male	4	68.7 ±3.5	351.5 ±19.8	0.592 ±0.119	210.5 ±13.4	545.2 ±70.1	0.612 ±0.045
S. collingwoodi	Female	2	59.9 ±6.91	325.5 ±25.5	0.336 ±0.146	226.6 ±21.7	507.5 ±44.5	0.562 ±0.019
	Female	5	47.3 ±6.44	300 ±0	0.328 ±0.039	227 ±7.3	670.6 ±27.9	0.738 ±0.099
***Siler* sp. 1**	Male	1	66.6	399	0.758 ±	227.6	410	0.635
***Siler* sp.2**	Female	2	67 ±2.24	300 ±0	0.291 ±0.073	261.7 ±9.7	698.5 ±1.5	0.377 ±0.043
***Siler* sp. 4**	Female	1	64.2	396	0.566	226.5	698	0.545
	Female	1	14.2	300	0.57	202.3	521	1.34
Telamonia climidiata	Male	1	79.9	300	0.37	197.9	401	0.23

Table 5.5. Spectral reflectance in the body region of dorsal carapace in heliophaninae and out group species.

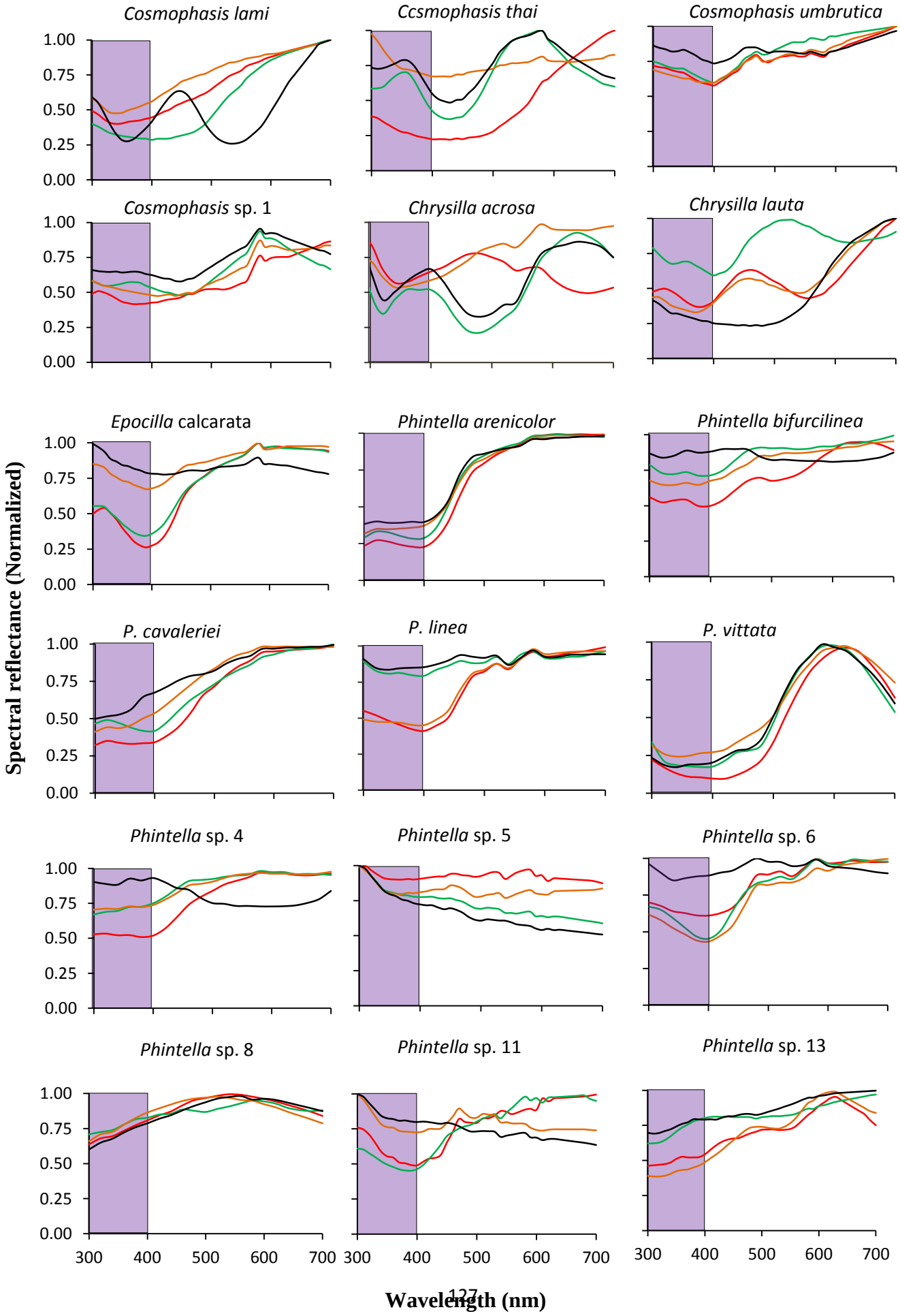
Species	Sex	N	Dorsal carapace					
			UV (300-400 nm)			VIS (400-700 nm)		
			Qt	Hue	Chroma	Qt	Hue	Chroma
Cosmophasis lami	Female	2	25.2 ±3.3	300 ±0	0.285 ±0.035	184.2 ±8.2	691 ±1	1.255 ±0.115
Cosmophasis thai	Female	1	61.4	355	0.46	207.7	580	0.91
Cosmophasis umbratica	Female	5	56.4 ±3.8	322.8 ±11	0.226 ±0.117	205.1 ±3.7	583.2 ±6.3	0.784 ±0.11
	Male	2	44.5 ±16.95	342.5 ±35.5	0.235 ±0.135	161.1 ±46.4	582 ±0	1.355 ±0.715
***Cosmophasis* sp. 1**	Female	2	68.6 ±2.5	300.5 ±0.5	0.23 ±0	260 ±1.6	684.5 ±10.5	0.47 ±0.02
Chrysilla acrosa	Female	20	56.3 ±2.14	300 ±0	1.128 ±0.038	156.7 ±2.3	675.8 ±2.4	1.405 ±0.034
	Male	20	55 ±2.57	310 ±6.8	1.094 ±0.105	155.6 ±6.6	661.5 ±7	1.285 ±0.101
Chrycilla lauta	Female	1	68.8	300	0.29	257.5	514	0.48
	Male	1	62.5	398	0.53	214.3	580	0.8
Epocilla calcarata	Female	1	61.2	300	0.46	151	557	0.72
	Male	1	69.7	302	0.49	191.1	533	1.01
P. arenicolor	Female	5	33.6 ±1.67	326.8 ±3	0.184 ±0.028	253.9 ±1.5	633.6 ±20	0.803 ±0.025
	Male	5	51.3 ±1.59	343 ±13.1	0.164 ±0.024	258 ±5	653.4 ±23.8	0.603 ±0.007
P. bifurcilinea	Female	5	74.1 ±2.26	336.4 ±15.4	0.15 ±0.086	269.8 ±7.4	662 ±34.9	0.34 ±0.053
	Male	5	70.7 ±5.88	397.2 ±0.6	0.325 ±0.069	275.2 ±3.2	521 ±44.6	0.231 ±0.043
P. cavaleriei	Female	5	45.5 ±2.43	315.4 ±1.7	0.192 ±0.02	239.3 ±5.4	694 ±3.1	0.741 ±0.047
	Male	5	34.8 ±4.22	329.8 ±18.4	0.226 ±0.048	231.6 ±6.5	653.1 ±27	0.841 ±0.051
P. clathrata	Female	1	85.5	300	0.27	204.3	400	0.28
P. linea	Female	5	81.9 ±1.88	300 ±0	0.147 ±0.015	269.3 ±5	639.6 ±32.4	0.246 ±0.029
	Male	5	77.8 ±6.86	391.1 ±2.4	0.16 ±0.023	272.5 ±4.6	529.4 ±62.1	0.268 ±0.052

P. vittata	Female	5	19.7 ±5.04	300 ±0	1.174 ±0.266	193.1 ±5.9	591.4 ±8.5	1.304 ±0.111
	Male	5	40.7 ±14.02	300.5 ±0.3	0.635 ±0.281	212.2 ±18.5	626.6 ±27.4	0.97 ±0.263
***Phintella* sp. 4**	Female	5	71.7 ±6.04	373.4 ±8.1	0.142 ±0.043	278.5 ±3	620.3 ±25.8	0.282 ±0.06
	Male	5	79.3 ±1.69	371.4 ±17.7	0.128 ±0.004	274.9 ±3	651.4 ±16.1	0.224 ±0.026
***Phintella* sp. 6**	Female	5	56.5 ±2.56	301.7 ±0.2	0.42 ±0.04	257.1 ±2.5	588.1 ±3.1	0.643 ±0.043
	Male	5	87.6 ±0.64	300 ±0	0.238 ±0.013	247 ±2.5	695.6 ±0.4	0.168 ±0.009
***Phintella* sp. 8**	Female	5	77.3 ±10.44	388.4 ±1.9	0.205 ±0.058	268.7 ±8.5	552 ±47.9	0.301 ±0.104
	Male	5	78.9 ±2.81	397.1 ±0.5	0.357 ±0.025	268 ±3	448.4 ±17.3	0.292 ±0.043
***Phintella* sp. 11**	Female	1	51.8	300	0.31	253.2	641	0.64
***Phintella* sp. 13**	Female	4	71.2 ±5.62	396 ±1.3	0.282 ±0.042	258.5 ±8.2	609.7 ±63.9	0.295 ±0.064
***Phintella* sp. 14**	Female	5	63.6 ±5.7	300 ±0	0.364 ±0.028	257.8 ±2.9	608.3 ±12.2	0.55 ±0.067
	Male	5	76.9 ±8.95	305.9 ±3.6	0.157 ±0.03	231.5 ±14.8	446.8 ±28.6	0.409 ±0.045
Orsima ichneumo	Female	1	61.2	300	0.46	151	557	0.72
	Male	1	69.7	302	0.49	191.1	533	1.01
Siler semiglaucus	Female	2	34.2 ±3.5	300.5 ±0.5	0.292 ±0.029	186.2 ±2.9	699.5 ±0.5	1.14 ±0.058
	Male	4	40.4 ±2.98	303.7 ±3.7	0.254 ±0.063	227.4 ±5.7	583.7 ±38.2	0.819 ±0.043
S. collingwoodi	Female	2	46 ±1.03	300.5 ±0.5	0.264 ±0.049	235.6 ±3.4	606.5 ±39.5	0.747 ±0.004
***Siler* sp. 1**	Female	5	52.1 ±6.26	301.4 ±1.1	0.298 ±0.074	234.4 ±3.8	554.6 ±6.2	0.72 ±0.069
	Male	1	35	300	0.432	232.1	525	0.934
***Siler* sp. 2**	Female	2	71.3 ±0.77	300 ±0	0.381 ±0.038	257.9 ±12	695 ±5	0.333 ±0.01
***Siler* sp.4**	Female	1	25.4	302	0.278	213.4	580	1.086
Telamonia climidiata	Female	1	84.8	311	0.14	278	580	0.19
	Male	1	48.8	300	0.68	232.1	676	0.88

Table 5.6. Spectral reflectance in the body region of lateral carapace in in heliophaninae and out group species.

Species	Sex	N	Lateral carapace					
			UV (300-400)			VIS (400-700)		
			Qt	Hue	Chroma	Qt	Hue	Chroma
Cosmophasis lami	Female	2	46.2 ±10.8	306 ±6	0.525 ±0.275	198.2 ±37.7	699 ±0	0.94 ±0.39
Cosmophasis-Thai	Female	1	72.7 ±	348 ±	0.33 ±	225.6 ±	580 ±	0.68 ±
Cosmophasis umbratica	Female	5	65.16 ±5	317.2 ±7.9	0.16 ±0.061	229.1 ±6.2	581.8 ±6.9	0.582 ±0.122
	Male	2	56.65 ±6.3	345 ±34	0.09 ±0.01	194.5 ±15.2	582 ±0	0.755 ±0.165
***Cosmophasis* sp. 1**	Female	2	81.6 ±9.5	300 ±0	0.16 ±0.03	252.1 ±7.5	696.5 ±3.5	0.32 ±0.06
Chrysilla acrosa	Female	20	69.95 ±1.2	300 ±0	1.138 ±0.06	175.1 ±1.4	666.1 ±4.9	1.003 ±0.045
	Male	20	60.74 ±1.8	305 ±5	1.346 ±0.069	150.8 ±4.9	678.6 ±4	1.401 ±0.068
Chrycilla lauta	Female	1	32 ±	300 ±	0.54 ±	156.2 ±	699 ±	1.48 ±
	Male	1	43.2 ±	300 ±	1.15 ±	184.7 ±	512 ±	1.22 ±
Epocilla calcarata	Female	1	75.5	315	0.44	200.3	700	0.87
	Male	1	33.3	300	1.24	130.8	699	1.89
P. arenicolor	Female	5	39.7 ±5.9	337.7 ±13.6	0.19 ±0.031	39.7 ±5.9	337.7 ±13.6	0.19 ±0.031
	Male	5	58.86 ±10	346.8 ±15.8	0.196 ±0.019	244.5 ±19.2	562.4 ±52.3	0.598 ±0.104
P. bifurcilinea	Female	5	88.24 ±2.5	352.4 ±18.4	0.155 ±0.013	253.2 ±5.4	521.1 ±61.7	0.256 ±0.017
	Male	5	63.82 ±8.4	397.4 ±1.8	0.364 ±0.06	272.4 ±3.6	501.2 ±26.5	0.329 ±0.059
P. cavaleriei	Female	5	56.96 ±7.5	387.2 ±6.7	0.402 ±0.102	265.2 ±6.5	664.8 ±12.2	0.411 ±0.102
	Male	5	41.88 ±4	391.3 ±3.7	0.397 ±0.072	244.5 ±7.6	692.1 ±1.7	0.641 ±0.052
P. clathrata	Female	1	83.5 ±0	300 ±0	0.34 ±0	179.8 ±0	401 ±0	0.37 ±0
P. linea	Female	5	85.9 ±2.7	316.9 ±16.9	0.105 ±0.01	276.2 ±4.7	569.1 ±35.1	0.188 ±0.016
	Male	5	79.5 ±3.5	395.6 ±0.6	0.254 ±0.008	282.2 ±5.9	542.8 ±40.9	0.162 ±0.038
P. vittata	Female	5	19.16 ±2.9	319.7 ±19.4	0.442 ±0.1	195.4 ±3.7	592.2 ±6.7	1.24 ±0.077

	Male	5	37.14 ±6.5	339.2 ±24	0.323 ±0.121	219.8 ±13.5	400 ±0	0.836 ±0.166
***Phintella* sp. 4**	Female	5	92.08 ±2	349.7 ±20.6	0.153 ±0.047	234 ±13.7	476.4 ±56.8	0.384 ±0.107
	Male	5	86.44 ±1.2	395.3 ±0.1	0.169 ±0.013	246.3 ±11.8	430.6 ±8.3	0.344 ±0.058
***Phintella* sp. 6**	Female	5	89 ±0.5	300 ±0	0.136 ±0.006	283.9 ±0.7	474.5 ±0	0.13 ±0.005
	Male	5	57.34 ±1	308.2 ±1.8	0.224 ±0.009	261.4 ±0.9	580 ±0	0.563 ±0.016
***Phintella* sp. 8**	Female	5	70.86 ±7.3	392.5 ±2.3	0.282 ±0.029	276.3 ±3.1	509 ±26.5	0.273 ±0.055
	Male	5	74.34 ±6.4	395.7 ±2.1	0.28 ±0.054	266.2 ±3	485.2 ±21.3	0.342 ±0.03
***Phintella* sp. 11**	Female	1	87 ±0	300 ±0	0.23 ±0	216.2 ±0	401 ±0	0.24 ±0
***Phintella* sp. 13**	Female	4	75.07 ±5.7	391.2 ±4.5	0.182 ±0.008	270.4 ±7.2	670.7 ±16.4	0.27 ±0.074
	Female	5	78.4 ±5.5	300 ±0	0.215 ±0.013	267.7 ±4.7	612.4 ±35.3	0.282 ±0.084
***Phintella* sp. 14**	Male	5	90.16 ±1.8	300 ±0	0.208 ±0.046	228.1 ±21.7	429.4 ±18	0.275 ±0.024
	Female	1	75.5	315	0.44	200.3	700	0.87
Orsima ichneumo	Male	1	33.3	300	1.24	130.8	699	1.89
	Female	2	54.1 ±19.7	300.5 ±0.5	0.311 ±0.017	205 ±39.8	699.5 ±0.5	0.855 ±0.474
Siler semiglaucus	Male	4	31.66 ±4	314 ±14	0.185 ±0.031	186.5 ±13.4	698.2 ±0.7	1.153 ±0.208
S. collingwoodi	Female	2	53.61 ±13.1	300.5 ±0.5	0.233 ±0.008	207.3 ±18.9	699 ±1	0.596 ±0.091
	Female	5	65.58 ±5.2	300 ±0	0.257 ±0.048	244.1 ±11.3	627 ±29.1	0.469 ±0.09
***Siler* sp. 1**	Male	1	15.3 ±	300 ±	0.33 ±	140.8 ±	697 ±	1.859 ±
***Siler* sp. 2**	Female	2	68.71 ±6.4	350.5 ±49.5	0.303 ±0.021	270.4 ±5.1	540 ±100	0.241 ±0.099
***Siler* sp. 4**	Female	1	28.72 ±	300 ±	0.211 ±	197.7 ±	698 ±	1.081 ±
	Female	1	65.1	300	0.34	258.4	634	0.5
Telamonia climidiata	Male	1	41.8	318	0.77	242.6	641	0.92


Cosmophasis lami
Ccsmophasis thai
Cosmophasis umbrutica
Cosmophasis sp. 1
Chrysilla acrosa
Chrysilla lauta
Epocilla calcarata
Phintella arenicolor
Phintella bifurcilinea
P. cavaleriei
P. linea
P. vittata
Phintella sp. 4
Phintella sp. 5
Phintella sp. 6
Phintella sp. 8
Phintella sp. 11
Phintella sp. 13
1.00
0.75
0.50
0.25
0.00
300
400
500
600
700
Spectral reflectance (Normalized)
Wavelength (nm)

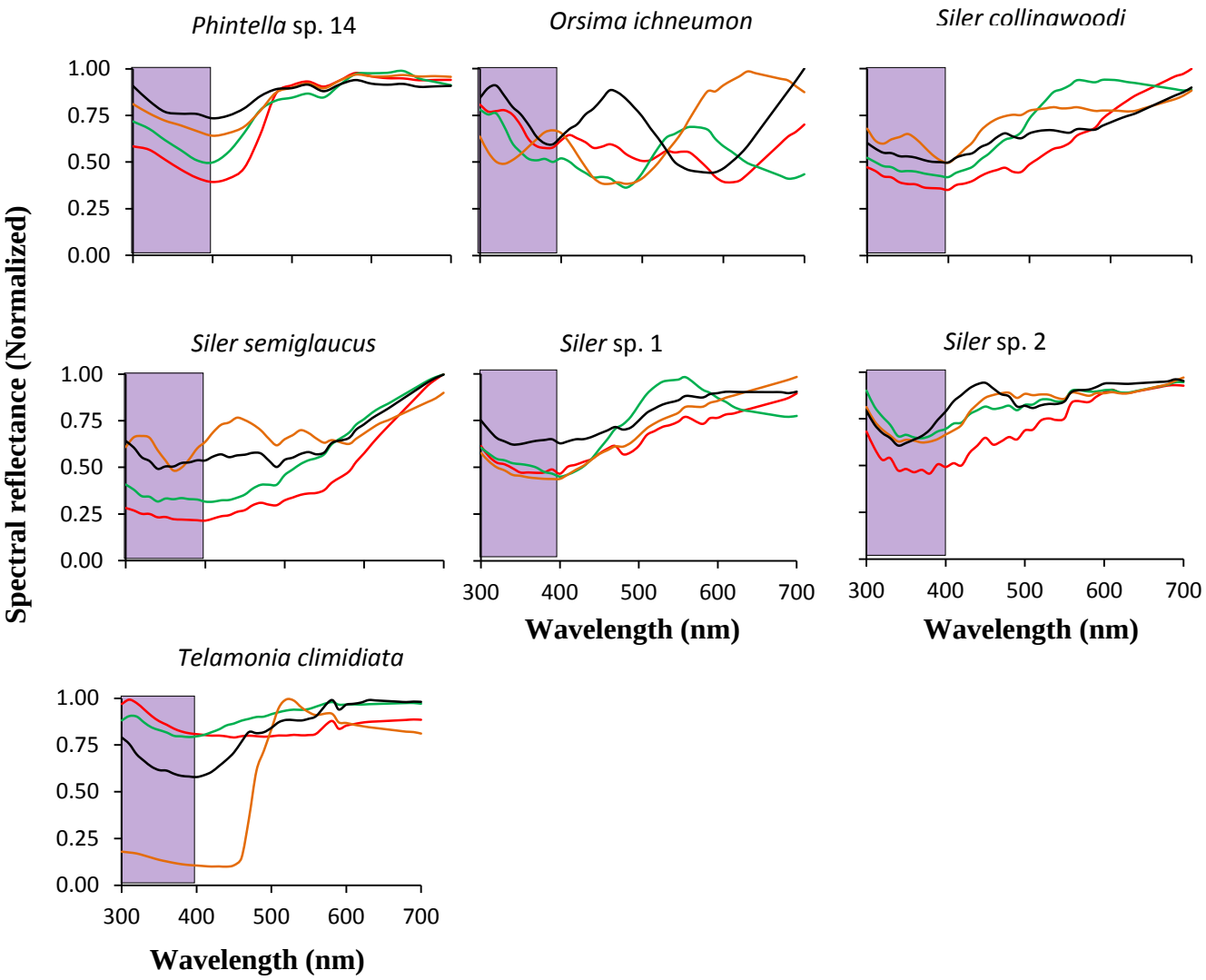


Figure 5.1. Normalized reflectance spectra of four body regions of females (red: dorsal abdomen, green: dorsal carapace, yellow: lateral abdomen and black: lateral carapace) across UV (shaded area, wavelength: 300-400 nm) and VIS (unshaded area, wavelength: 400-700 nm) in 25 species of heliophanines.

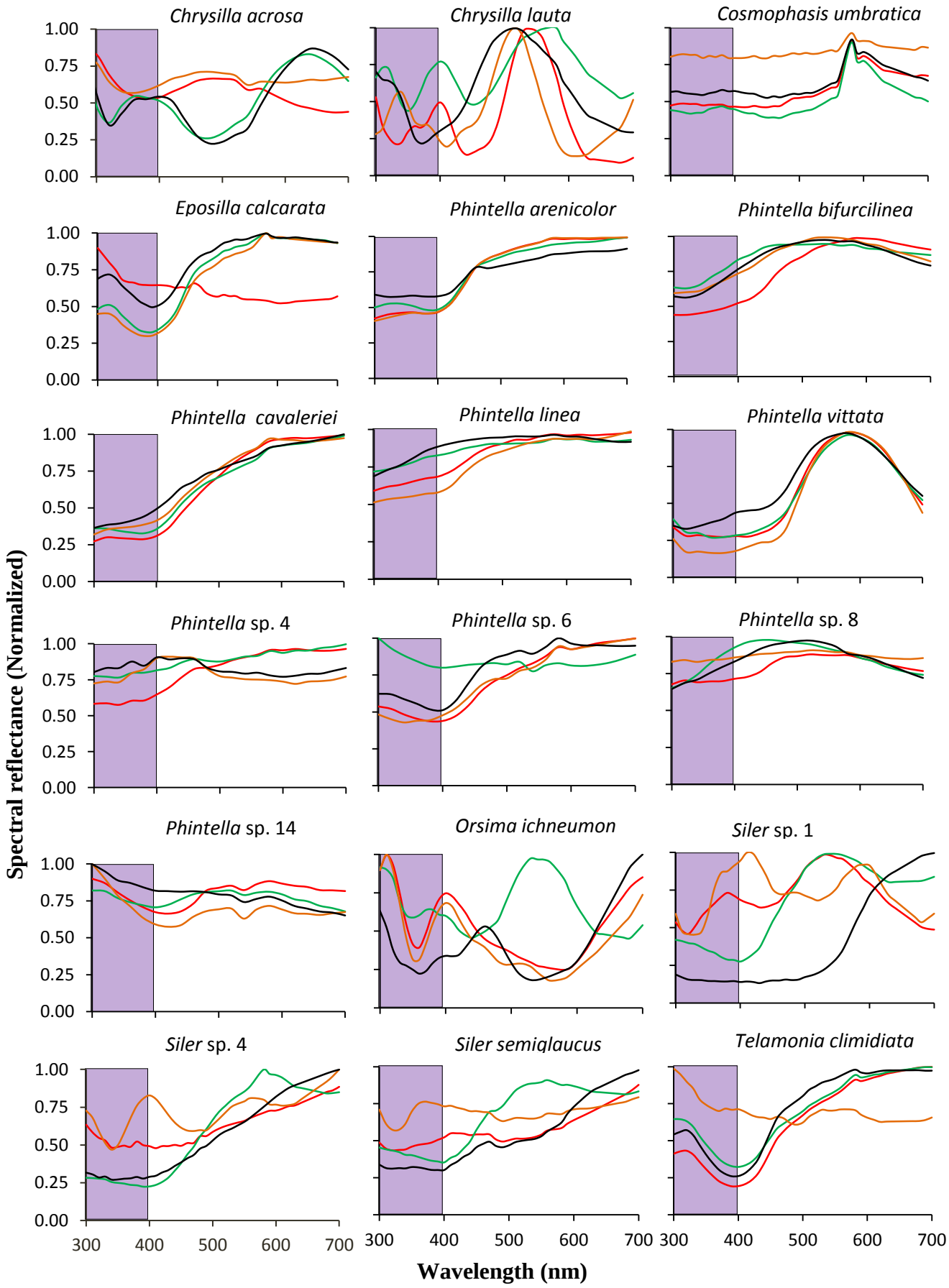


Figure 5.2. Normalized reflectance spectra of four body regions of males (red: dorsal abdomen, green: dorsal carapace, yellow: lateral abdomen and black: lateral carapace) across UV (shaded area, wavelength: 300-400 nm) and VIS (unshaded area, wavelength: 400-700 nm) in 18 species of heliophanines.

5.3 Result

5.3.1 Phylogeny

The ML and BI analyses of the combined data produced best trees with slightly different topologies, but they agree on heliophanine monophyly (**Fig. 5.3**). The phylogenetic topologies also recover *Phintella* and *Siler* as monophyletic, but do not recover *Chrysilla, Cosmophasis* and *Orsima* as monophyletic; *Orsima* nests within *Chrysilla*; *Cosmophasis* is paraphyletic.

5.3.2 Sexual dichromatism in UV and VIS colour

Figure 5.4 visually traces the evolution of sexual dichromatism in the UV and VIS colours. It indicates that sexual dichromatism is widespread (11 out of 22 species) in heliopahnines and that the evolution of sexual dichromatism in the UV and VIS colour shows the similar patterns. Furthermore, closely related species usually showed similar sexual dichromatism. These results imply that UV and VIS colour most likely often evolve together in the evolutionary history.

5.3.3 Colour evolution

There was no obvious evolutionary trend in UV total brightness (Qt) of both dorsal abdomen and dorsal carapace (**Fig. 5.5**). In other words, the total brightness in the UV increases or decreases at random and varies among these species. However, UV hue and UV chroma showed obvious evolutionary trend (**Figs. 5.6 and 5.7)**. The UV hue tended to increase from basal to derived species in the genus *Phintella*, but the UV hue tended to decrease from basal to more derived species in the clade *Cosmophasis* + *Chrysilla* + *Orsima* in both body regions **(Fig. 5.6).** The UV chorma showed converse trend to the UV hue (**Fig. 5.7**). Whenever it showed a decreasing trend in the UV hue,

the UV chroma usually exhibited an increasing trend in the same clade. In addition, the evolution of the UV chroma had similar trends for both dorsal abdomen and dorsal carapace.

The evolution of the VIS colours showed different evolutionary patterns from UV colour. Different clades showed different increasing or decreasing in the total brightness of VIS on both body regions (**Fig. 5.8**). In the clade of *Siler*, the VIS brightness was relatively stable for both dorsal abdomen and carapace. However, the evolution of VIS brightness in *Phintella* showed a trend of increase in both body regions. In contrast, the evolution of VIS brightness of both body regions tended to decrease within the clade *Cosmophasis* + *Chrysilla* + *Orsima*. However, there was no clear evolutionary trend in the VIS hue of both body regions (**Fig. 5.9**). Similar to the evolution of UV colour, the evolution of both total VIS brightness and VIS chroma showed contrast trends; the total VIS brightness showed a decreasing evolutionary trend in the clade of *Cosmophasis* + *Chrysilla* + *Orsima* (**Fig. 5.8**), however, VIS chroma in this clade tended to increase (**Fig. 5.10**). The total VIS brightness showed a decreasing trend, but the VIS chroma tended to decrease in *Phintella* (**Fig. 5.10**).

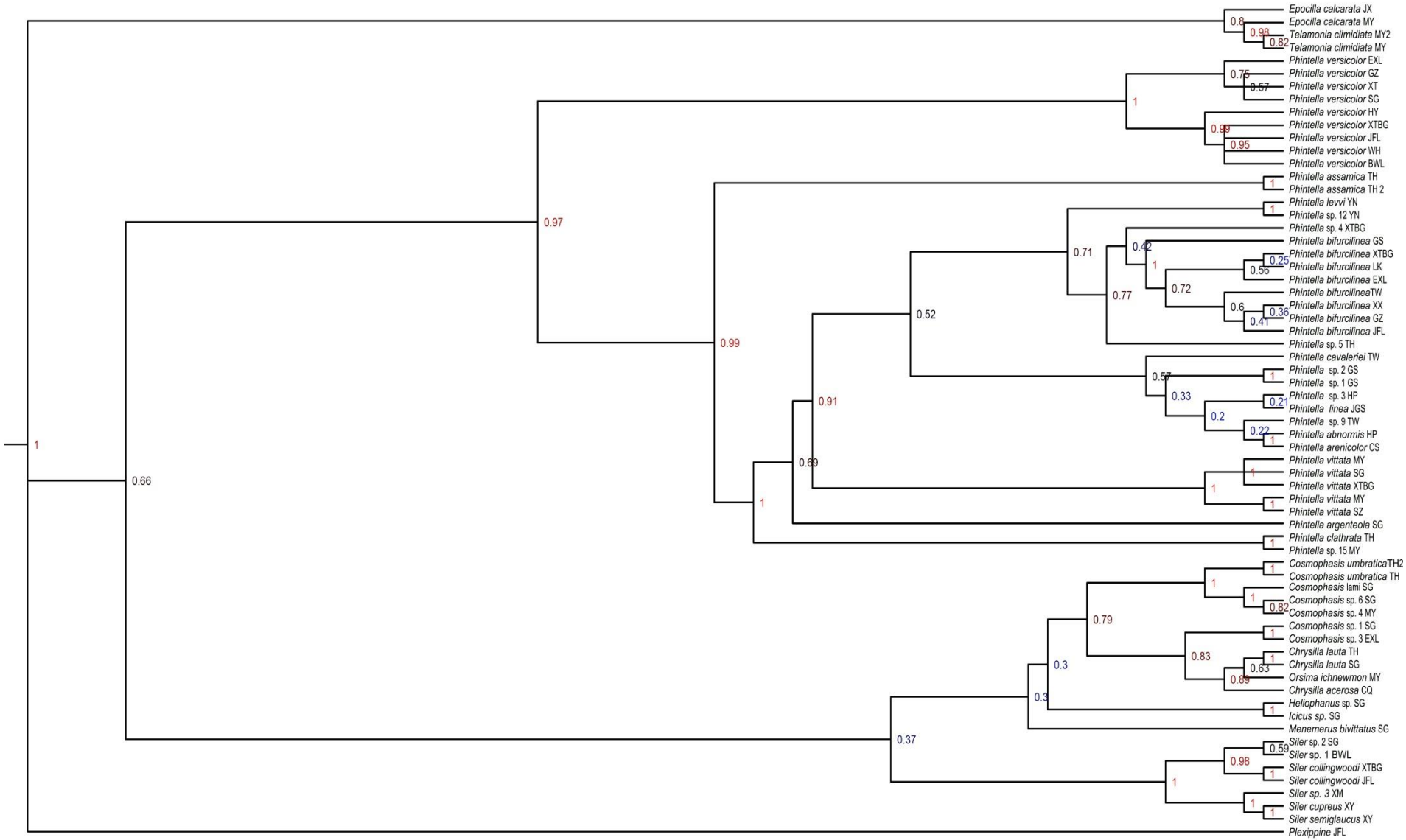


Figure 5.3. Phylogeny of heliophanine salticid spiders based maximal likelihood analaysis (ML). Numbers in red indicate bootstrap supports $\geqslant$ 0.75, numbers in brown indicate bootstrap supports between 0.50 and 0.75; and numbers in blue indicate bootstrap supports < 0.50).

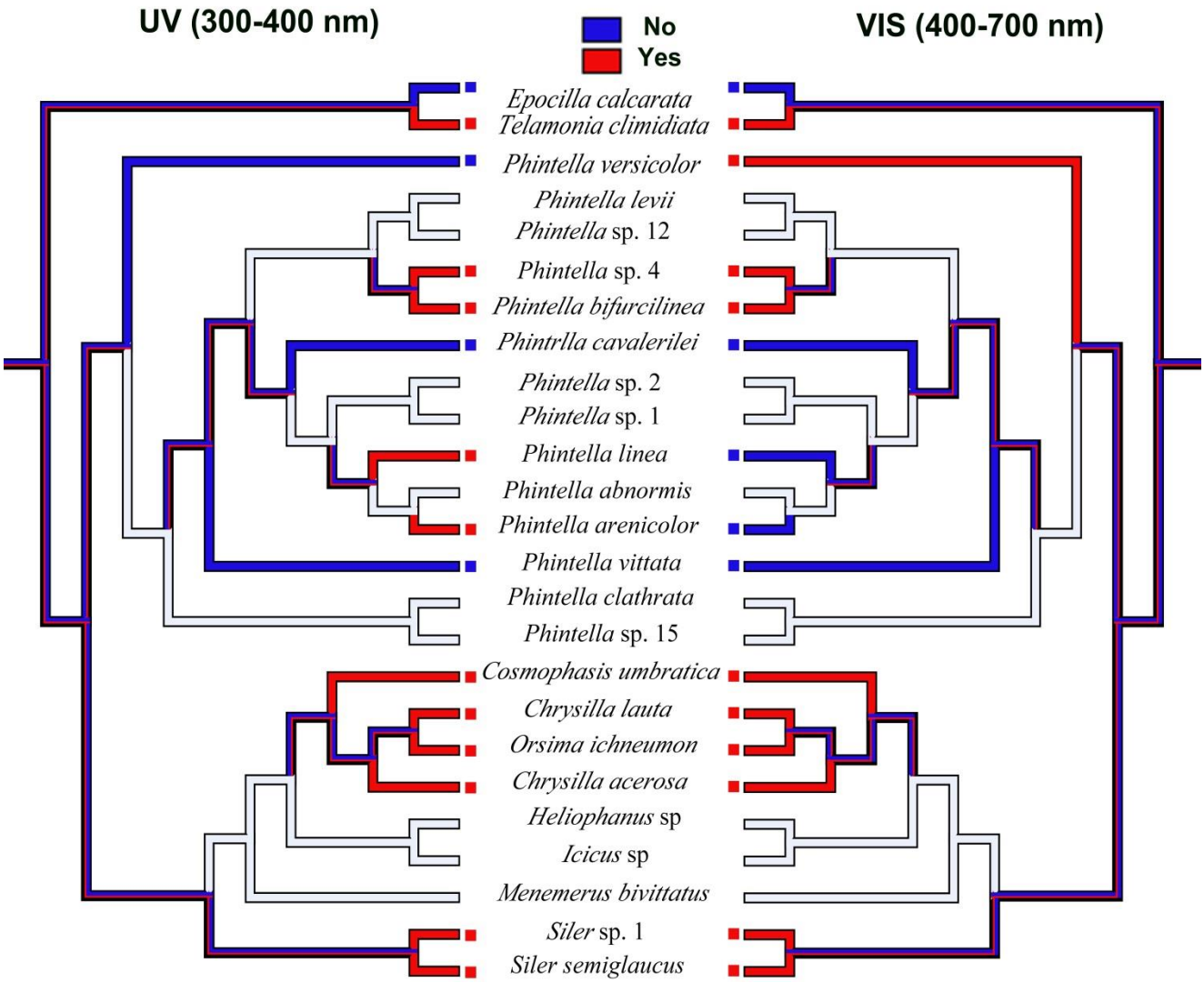


Figure 5.4. Reconstructed evolution of sexual dichromatism in the UV and VIS reflection of heliophanines on the best ML tree. Blue: absence of sexual dichromatism (No); red: presence of sexual dichromatism (Yes); and grey: unknown.

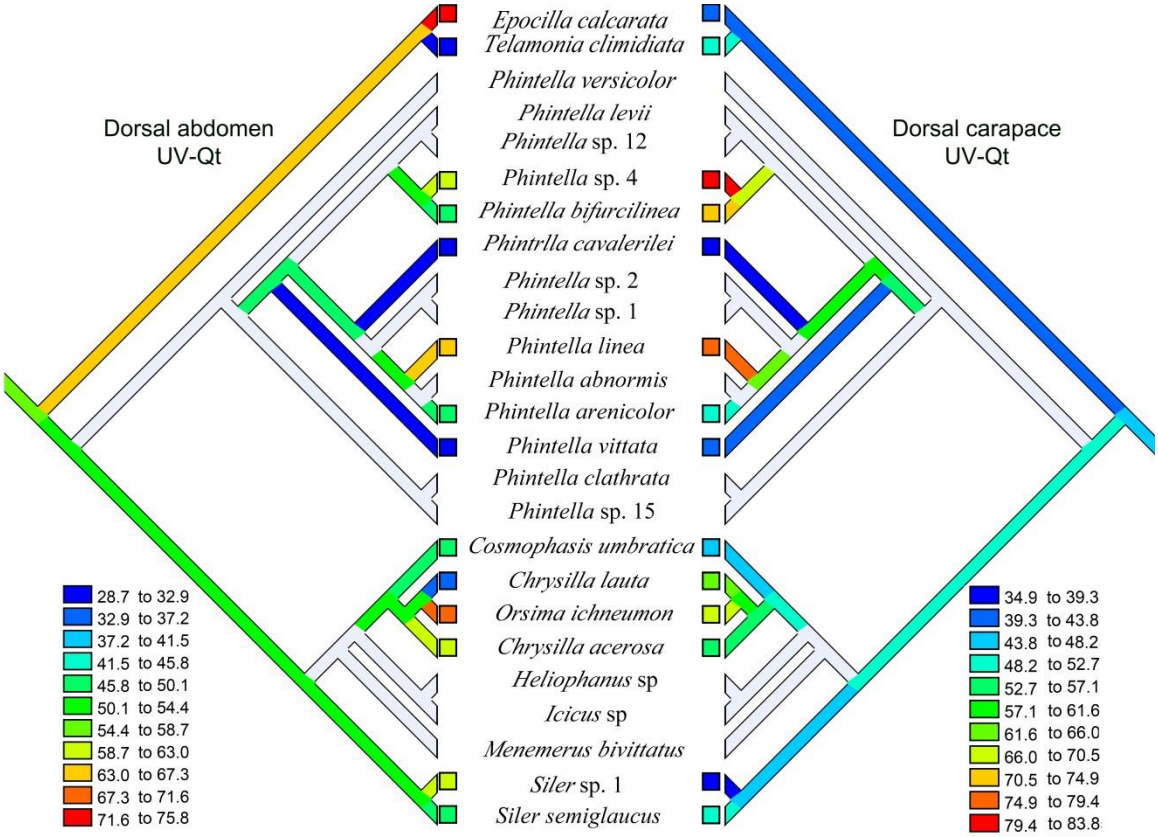


Figure 5.5. Reconstructed evolution of the UV total brightness (Qt) of dorsal abdomen and dorsal carapace in male heliophanines. Colours donate UV total brightness classes.

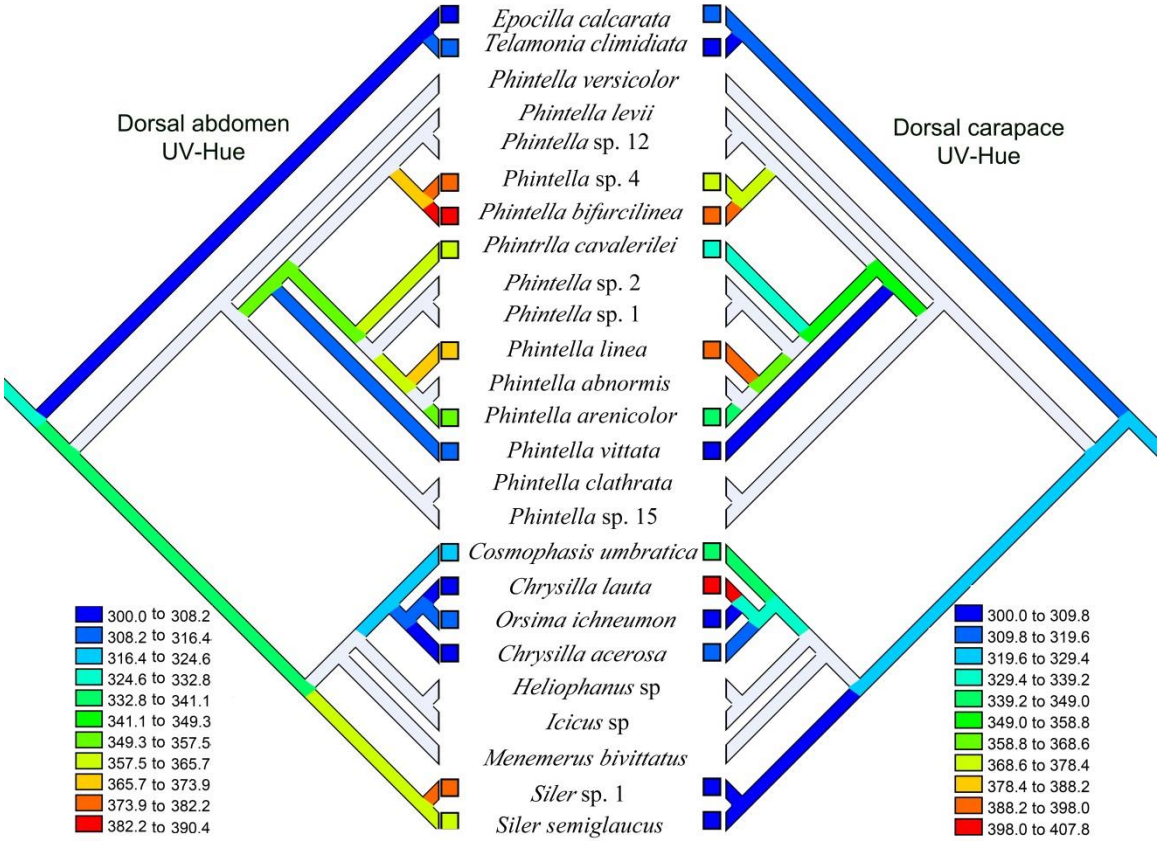


Figure 5.6. Reconstructed evolution of the UV hue (nm) of dorsal abdomen and dorsal carapace in male heliophanines. Colours donate UV hue classes.

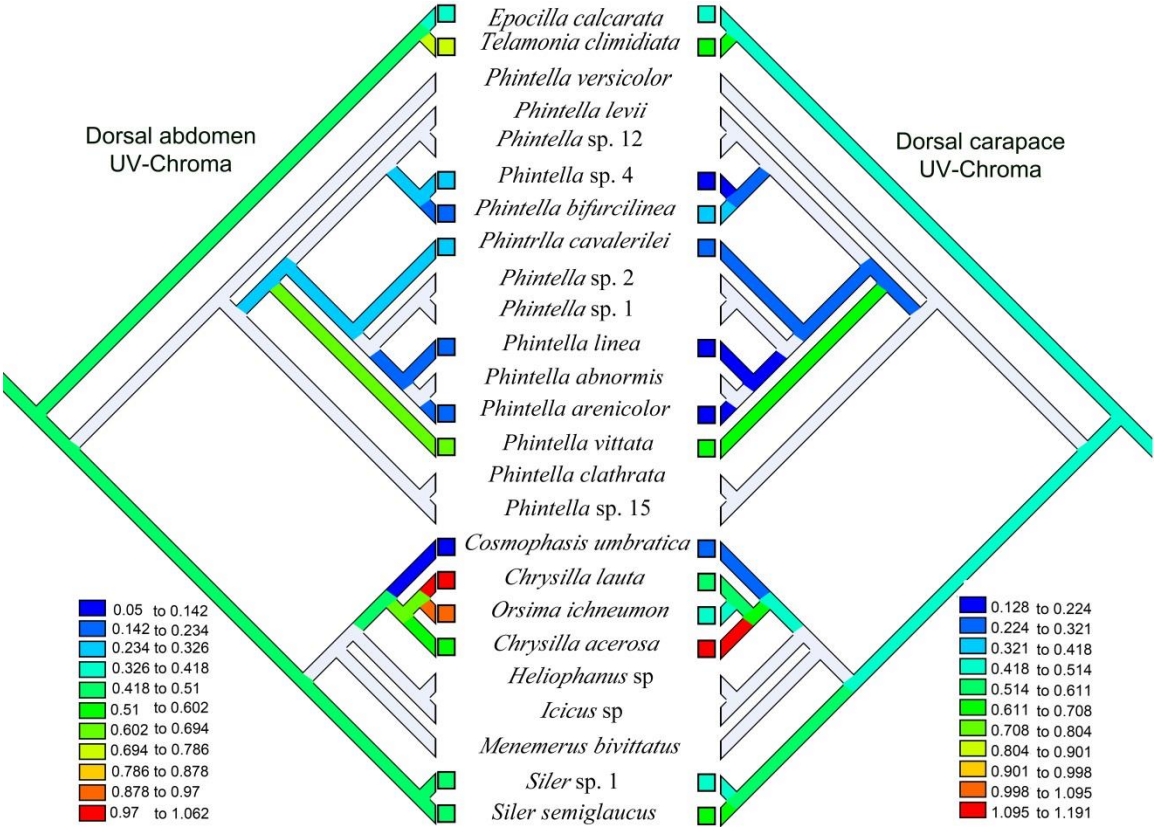


Figure 5.7. Reconstructed evolution of the UV chroma of dorsal abdomen and dorsal carapace in male heliophanines. Colours donate UV chroma classes.

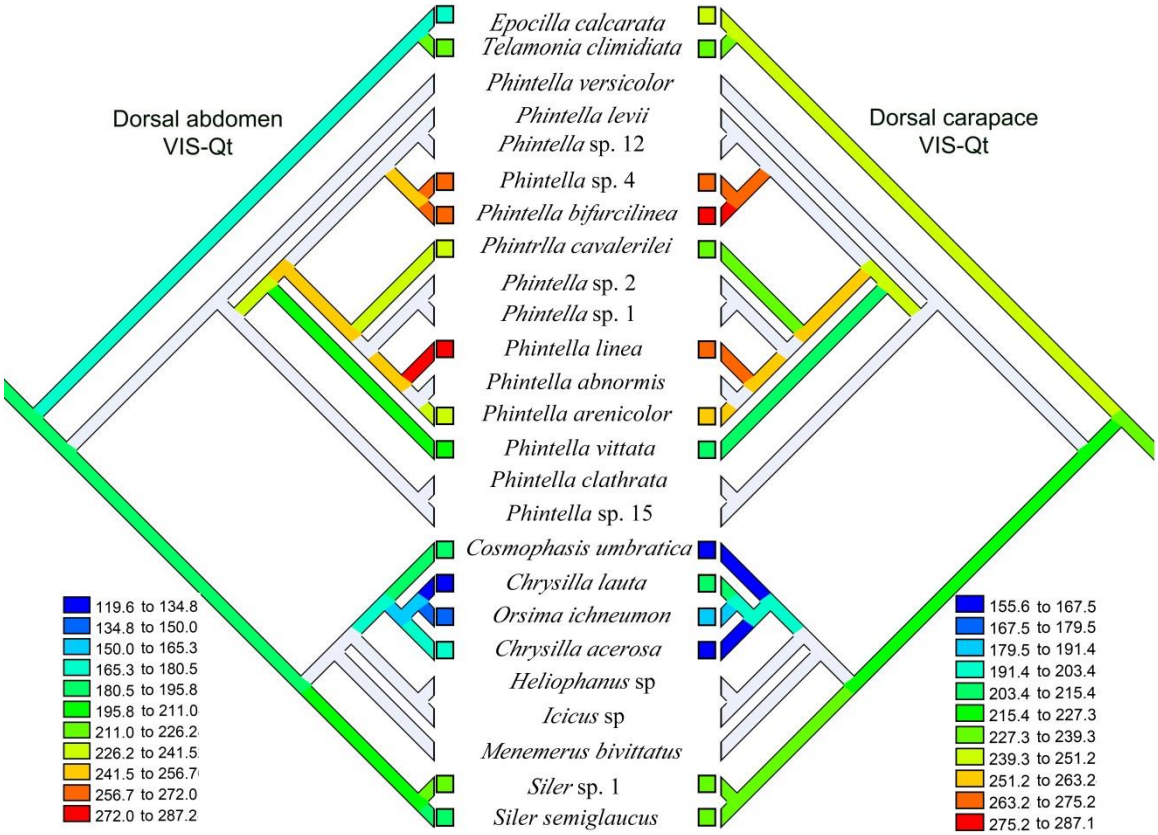


Figure 5.8. Reconstructed evolution of the VIS total brightness (Qt) of dorsal abdomen and dorsal carapace in male heliophanines. Colours donate VIS total brightness classes.

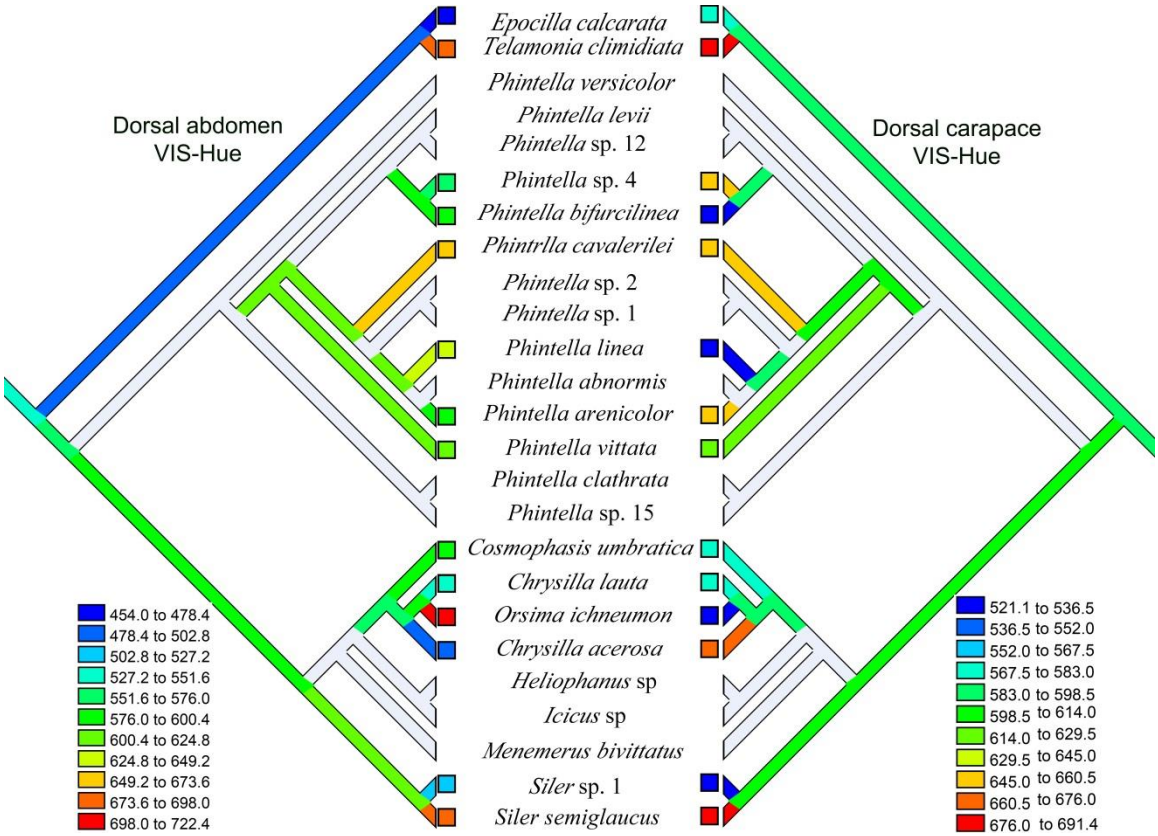


Figure 5.9. Reconstructed evolution of the VIS hue (nm) of dorsal abdomen and dorsal carapace in male heliophanines. Colours donate VIS hue classes.

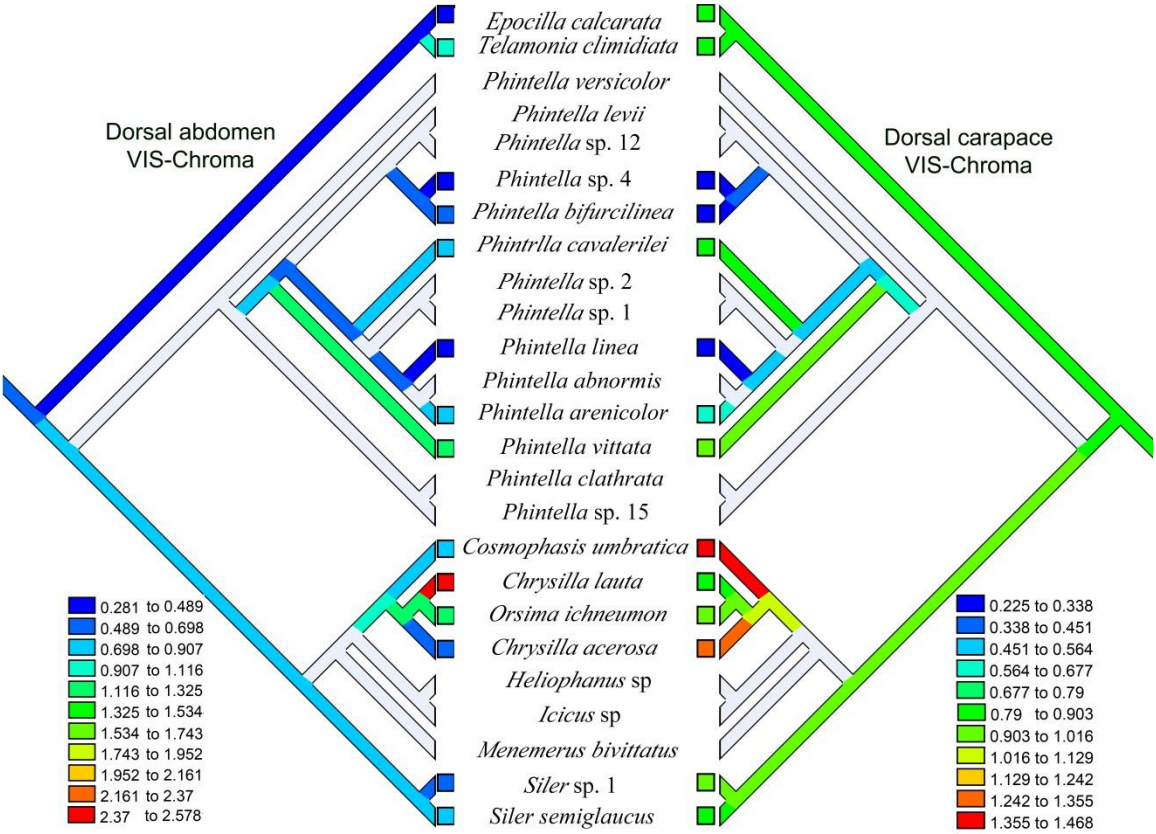


Figure 5.10. Reconstructed evolution of the VIS chroma of dorsal abdomen and dorsal carapace in male heliophanines. Colours donate VIS chroma classes.

5.4 Discussion

Our results showed that sexual dichromatism is common in both UV and VIS colours, but scattered in heliophanine spiders, suggesting a possible convergent evolution of sexual dichromatism. This widespread of sexual dichromatism in both UV and VIS colours as a result of sexual selection, mostly female mate choice, is probably contributing to the high species diversity of the heliophenine salticids, particularly the genus *Phintella*. It has long been assumed that sexual selection promotes speciation as many closely related species differ in sexual ornaments but are otherwise very similar (Danley and Kocher, 2001; Darwin, 1871; West-Eberhard, 1983). It is also predicted that strong sexual selection promotes speciation involving two steps: (1) stronger sexual selection may cause more evolutionary changes in ornaments; and (2) changes in ornaments may cause reproductive isolation, i.e., speciation (Cardoso and Mota, 2008). We also found that sexual dichromatism in UV and VIS colour occurs in the same species of heliophanines. This suggests that UV can be as common as VIS in salticids, and there is nothing unusual for these species to use UV as a clue in mating choice or intrasexual competition. However, we do not mean that UV colour is more important than VIS colours.

Heliophanine species seem to have evolved to increase either chroma or total brightness in the same clade for both UV and VIS colours. The total brightness and chroma usually showed a reversal evolutionary trend. That is, in a given species, heliophanine that gains a higher chroma usually may lower the total brightness or vise versa. For example, species of *Phintella* tended to increase brightness from basal to more derived species, but decrease chroma at the same time, however, species of other clades (*Cosmophasis*, *Chrysilla* and *Orsima*) evolve to decrease brightness but to increase chroma. This implies that the species may enhance their inter- or

intrasexual communication efficiency by increasing the total brightness in general or increasing the purity of a specific narrow range of spectral wavelength. This finding is probably as a result of the evolutionary mechanism under Fisher's runaway theory (Fisher, 1915).

Our findings suggest that the evolution of UV hue may be also related to speciation events. The UV hue originally at a short wavelength, as it evolved to either longer or even much shorter wavelength in different clades. However, the reversion can be happened occasionally in the evolution of hue such as in *P. vittata*. This species closely related to the rest of the other *Phintella* species, yet it showed similar spectral reflectance with the other species. This repeatedly shift in the hue has been found in the species *Dendrobates pumilio* as well (Wang and Shaffer, 2008). However, once this shift has happened, the species would have diverged from the other species in the same clade.

However, heliophanine salticids showed no clear evolutionary pattern in the VIS hue. Perhaps the limited number of species and the male colouration is unknown in this study are not allowed to trace the evolution of a wide range of spectral wavelength (400-700 nm). However, in most closely related species, the VIS hue usually showed a remarkable difference, which is very different with the evolution of total VIS brightness or VIS chroma. Therefore, the contrast direction divergence of hue may be contributing to the origin of speciation events in this clade.

Our sexual dichromatism in the UV of *P. vittata* is inconsistent with previous study (Li et al., 2008a; Li et al., 2008b), which showed strong sexual dichromatism in several main body parts in UV. We did not achieve the same result in this study, this may be because the difference in the analysed spectral reflectance range. In this study,

we analysed the UV only between 300 and 400 nm for all the species, but *P. vittata* shows a remarkable peak at around 285 nm in the UV in the early study (Li et al., 2008a). However, the UV-blocking mating choice experiments conducted in **Chapter 3** revealed the same female mate choice as in the early study (Li et al., 2008b).

We only analysed spectral reflectance as UV and VIS, it thus may not be allowed a fully understanding of evolutionary history of colouration. Therefore, to investigate the evolutionary process of colouration, more studies are required to divide the spectral wavelengths into more narrow ranges (at least in VIS band). In addition, limited number of species have been involved in this study, therefore many missing data need to be filled by colours from more species of this clade to confirm the evolutionary patterns.

CHAPTER 6

General conclusions and future research directions

6.1 General conclusions

Sexual dichromatism was reviewed in **Chapter 1**, and based on the previous studies of sexual dimorphism (Agrawal, 2001; Arnqvist, 1998; Arnqvist et al., 2000a; Richman and Jackson, 1992; Ritchie, 2007b; Roulin and Bize, 2007), I hypothesised that sexual dimorphic colouration (including UV) may have evolved through sexual selection in jumping spiders. To test this hypothesis I first quantified body colouration of multiple closely related species and then conducted a series of behavioural experiments in these species. To understand the origin and evolution of UV and VIS colours in these species, I reconstructed (collaborated with Dr Kathy Su) the molecular phylogeny of the subfamily of Heliophaninae and then traced back the evolutionary process of colours by mapping colour to the tree. In this final chapter, I will summary the main findings and make the conclusions.

Previous studies suggested UV reflectance may be widespread and important in female mate-choice in jumping spiders (De Voe, 1975b; Land, 1969b), but evidences only have come from the studies of two species, *Cosmorphasis umbratica* (Lim and Li, 2006a; Lim et al., 2007b) and *Phintella vittata* (Li et al., 2008b). I thus in **Chapter 2** investigated variations in spectral reflectance across UV (300-400 nm) and human visible (VIS) (400-700 nm) wavelength ranges between species, between body regions and between sexes in nine species of the jumping spider genus *Phintella* from Asia. Particularly, intersexual colouration was compared in details based on four main body regions (dorsal carapace, lateral carapace, dorsal abdomen and lateral abdomen) and three colour matric (total brightness (the total area under the spectral reflectance curve within a specific range of spectral reflectance), hue (wavelength at which the spectral reflectance value reaches max in a specific reflectance range), and chroma (purity of colours)). I found that: (1) UV reflectance commonly existed in

these nine species and in both male and female; (2) There were considerable variations in spectral reflectance between species, between body regions and between sexes; (3) The variations of UV reflectance range were usually different from VIS reflectance range; (4) All the nine species showed sexual dichromatism in spectral reflectance in both UV and VIS spectral ranges; and (5) The variations in the total brightness (Qt), hue and chroma were usually not consistent with each other. This is the first comparative study of inter- and intra-specific variations in spectral reflectance across UV and VIS ranges in jumping spiders. I speculate that both sexual selection and natural selection may play a role in the evolutionary of *Phintella* body colouration and that the total brightness and chroma may have evolved faster than the hue.

The remarkable diversity of sexual dimorphic colouration and species present in salticids has been considered the consequence of sexual selection. UV reflection is most probably widespread in salticids and is known to be used female mate-choice in a few species (Lim and Li, 2006a, b; Lim et al., 2007, 2008a, b; Li et al., 2008a, b). Surprisingly, however, studies of functioning of UV reflection across multiple closely related species are scarce. Moreover, although UV reflection is not uncommon in females, the role of UV in male mate-choice in animals remains poorly understood. In **Chapter 3**, I conducted a series of behavioural experiments to investigate the role of UV reflection in female mate-choice and male mate-choice of eight closely related species, all of which were from the genus of *Phintella*. In my experiments, the visual appearance of potential mates (either males or females) was manipulated using UV blocking (UV–) filters or without (UV+). I found that: (1) both females and males of two tropical species (*P. vittata* and *Phintella* sp. 8), but males of only one temperate species (*P. cavaleriei*) significantly preferred UV+ mates, suggesting that UV

reflection is more likely to be used in mate choice by tropical species; (2) both males and females of three species (*P. cavaleriei, P. vittata* and *Phintella* sp. 8) significantly or marginally significantly preferred UV+ mates, providing the first evidence of UV reflection in male mate-choice in jumping spiders and also indicating that the role of UV reflection in male mate choice is at least as crucial as in female mate-choice; (3) both males and females of *P. vittata* and *Phintella* sp. 8 significantly preferred UV+ mates, a first evidence of UV reflection in mutual mate-choice in jumping spiders; and (4) human-visible rather than UV reflection tended to be used in mutual mate choice in one species (*Phintella* sp. 14). This is the first study to investigate functioning of UV reflection in female mate-choice and male mate-choice across multiple closely related species in jumping spiders.

Sexual dichromatism is widespread across animal kingdom. It is, however, not uncommon in nature for a species in which both the sexes are brightly coloured, being sexually dichromatic in many body regions but sexual monochromatic in other body regions. How both sexually dichromatic and monochromatic colours coexist within a species remains largely unexplored. In **Chapter 4**, I addressed this using *Chrysilla acerosa,* a highly ornamented jumping spider in which both males and females have many body regions that are brightly coloured. I first quantified the colour of four main body regions (dorsal abdomen, lateral abdomen, dorsal carapace and lateral carapace) of males and females using spectrophotometer, and tested for the existence of sexual dimorphism in colour, including UV reflection. I found that all the body regions were sexually dichromatic in the UV and VIS wavelength range, or both except that their dorsal carapace was sexually monochromatic in both the UV and VIS ranges. I then conducted mating experiments by pairing individual males and virgin females under full-spectral lighting to test whether successful mating was associated with sexually

dichromatic or monochromatic colour of a specific body region. Surprisingly, the sexually monochromatic VIS colour of male dorsal carapace, but not the sexually dichromatic colours of other body regions, was the best predictor of mating success (likelihood of copulation). I then further compared the colours among the successful mated males, the males that have failed to mate, and females. I found that the successfully males had dorsal carapace colour that matched female dorsal carapace colour. This suggests that females prefer for colour-similar mates in *C. acerosa* and in other animals. This result is inconsistent with many previous studies that claimed that sexual dimorphic colours are indispensable in mate-choice (Clark and Uetz, 1993; Hill, 1990, 1991a; Houde, 1987). This female preference for more colour-similar males is in contradiction with the expected advantage as assumed by the 'genetic compatibility' hypothesis. I then investigated the direct and indirect benefits that connected to this sexual monomorphic colour-based mating decision. Results indicated that in *C. acerosa*, females obtained both direct and indirect benefits in terms of larger batch size (the number of eggs per egg-sac) and higher offspring survivorship when mating with males having similar dorsal carapace VIS chroma. This study suggests that more attention should be paid to sexually monomorphic colours in future studies of sexual selection.

Sexual dichromatism is widespread in jumping spiders of the subfamily Heliophaninae (Salticidae) and has been assumed the result of sexual selection. However, the evolution of sexual dimorphic colouration in heliophanines remains unexplored. In **Chapter 5**, I first reconstructed molecular phylogeny of Heliophaninae using five genes (16S/ND1, 28S, Actin 5C and COI), and then traced the evolution of sexual dichromatism in this clade. Furthermore, correlation between male colouration evolutionary process and speciation events has been analysed. Results revealed that:

(1) sexual dichromatism in the ultraviolet (UV: wavelength 300–400 nm) can be as common as in the human visible (VIS: wavelength 400–700 nm) in heliophanines examined; (2) sexual dichromatism in the UV and VIS have shown the similar evolutionary trends in the same species; (3) evolutionary changes of hue may be most probably correlated with speciation divergent; (4) the evolutionary changes in the total brightness (Qt) showed opposite direction from the evolutionary changes in chroma in both UV and VIS colours in the same clades. These results suggest that sexual dimorphic colouration may be a result of sexual selection through colour-based mating choice mechanism in Salticidae.

6.2 Future Directions

My results indicate that UV reflection commonly exists in the heliophanine salticids and sexual dichromatism in UV is also common in this species (**Chapter 2**). However, the function of UV in sexual selection remains unknown in many species of heliophanines tested except *Phintella vittata* (Li et al., 2008b), *Cosmophasis umbrutica* (Lim and Li, 2006a; Lim et al., 2008b), *Chrysilla acerosa* (**Chapter 4**) and other seven species of *Phintella* that were studied here (**Chapter 3**). Thus, more studies are needed to objectively evaluate the importance of UV in sexual selection in jumping spiders and the relative importance of UV and VIS colours in sexual selection.

Biologists usually interest in how females select mates based on male ornamentations, but less attention has been paid to male mate-choice based on the female ornamentations. The results from **Chapter 3** imply that colour-based male mate choice may be common in jumping spiders. Hence more studies should be conducted on male mate-choice in salticids and other invertebrates.

The role of sexually dimorphic traits (including colouration) in sexual selection has been intensively studied in animals (Berglund et al., 2005; Hill, 1991a; Iwasa and Pomiankowski, 1994; Johnstone, 1995; Nicoletto, 1995; Olsson, 1994; Robinson et al., 2014). However, some studies (Hager, 2001; Johnstone et al., 1996; Kraaijeveld et al., 2007; Summers et al., 1999) have demonstrated that sexually monomorphic traits in males can be vital in female mate-choices. However, these investigations have mainly focused on vertebrates, and few studies have been conducted in invertebrates. The results of **Chapter 4** showed that females preferred to mate with the males that exhibited similar colour with themselves rather than the

males that showed different colours. This suggests that female mate-choice do not have to rely on sexually dimorphic traits, and thus more studies should be conducted to investigate the role of sexually monomorphic traits in sexual selection in invertebrates.

The adaptive significance of sexually selected traits usually has been studied separately in sexual selection or in natural selection when refers to sexually selected traits. However, sexually selected traits may often result from the interactions of sexual selection and natural selection (Gorman et al., 2014; Price, 1998a), and it is necessary to take both natural and sexual adaptive significance into consideration in order to trace the evolution of these characters in future studies.

In order to trace back the evolutionary history of colour in jumping spiders, colouration from more species are required. As this study is the first investigation to try to study the evolution of colour, and the number of species used in this study is relatively small (**Chapter 5**), studies of spectral reflectance and the role of colouration in sexual selection from more species of salticids are needed in the future.

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